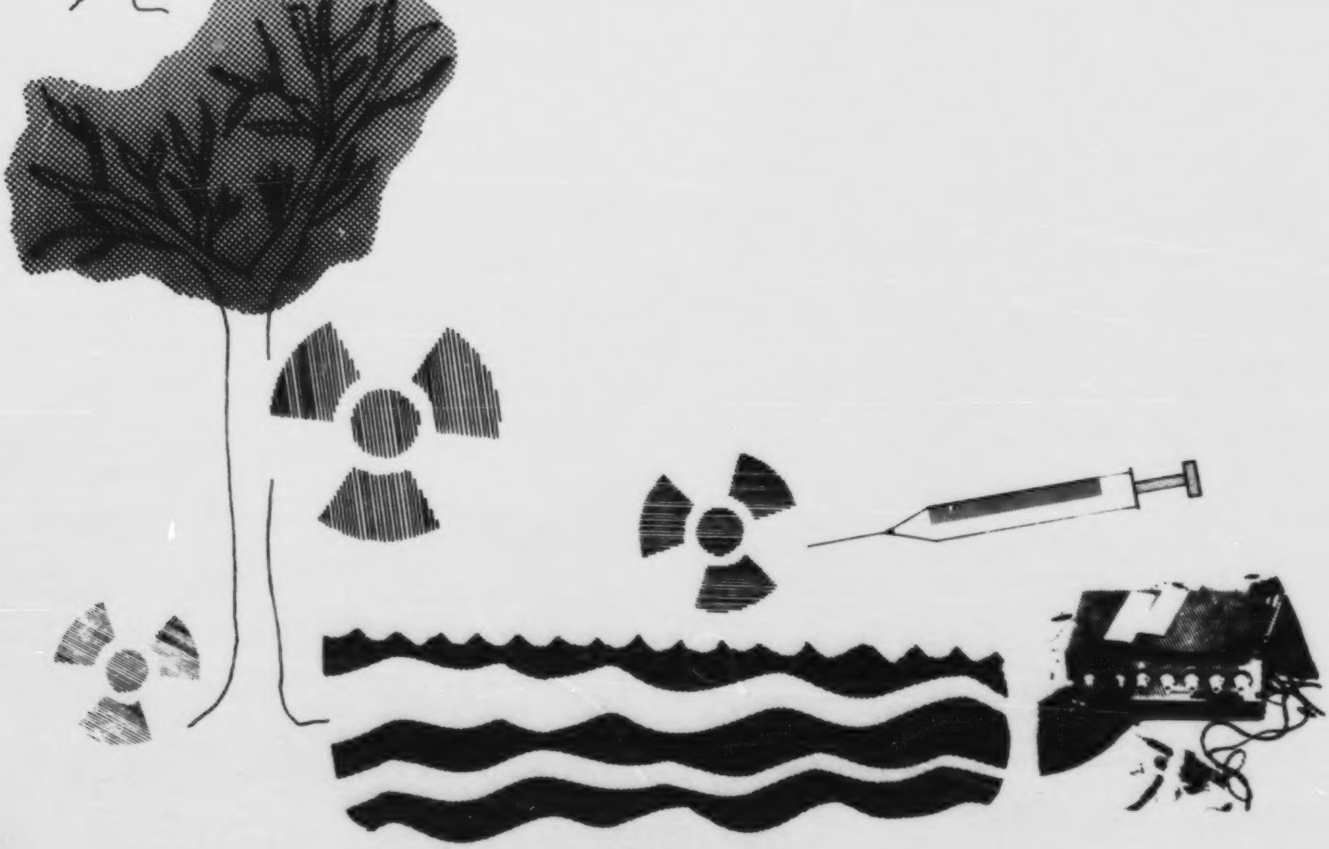
77-EHD-11

Health and Welfare Canada
Santé et Bien-être social Canada

Health Protection Branch
Direction générale de la protection de la santé

toxaphene



ENVIRONMENTAL HEALTH
CRITERIA DOCUMENT

TOXAPHENE

ENVIRONMENTAL HEALTH DIRECTORATE
HEALTH PROTECTION BRANCH
DEPARTMENT OF NATIONAL HEALTH AND WELFARE

SEPTEMBER 1977

77-EHD-11

COPIES OF THIS REPORT

CAN BE OBTAINED FROM:

INFORMATION DIRECTORATE

DEPARTMENT OF NATIONAL HEALTH
AND WELFARE

BROOKE CLAXTON BUILDING

OTTAWA K1A OK9

THIS DOCUMENT WAS PREPARED UNDER CONTRACT BY:

"IAN W. FRENCH & ASSOCIATES LIMITED"
SEPTEMBER 1977

THE REPORT IS REPRODUCED AS RECEIVED FROM THE CONTRACTOR. THE CONCLUSIONS AND RECOMMENDATIONS CONTAINED HEREIN REPRESENTS THE OPINION OF THE CONTRACTOR AND DO NOT NECESSARILY CONSTITUTE ENDORSEMENT BY THE DEPARTMENT OF NATIONAL HEALTH AND WELFARE.

INTRODUCTION

The purpose of this criteria document was to critically review the present knowledge available in the scientific literature to determine if a specific limit concentration standard for toxaphene in Canadian drinking was necessary, and, if so, to provide information relevant to determining the quantitative aspects of the standard.

Although all aspects of toxaphene were searched, including chemistry, uses, occurrences, metabolism, toxicity, etc., particular attention was given to reviewing data which provided information on the threshold dose which caused any effect in animals or man at the organ or cellular levels, as well as to the "no-effect" dose, especially as it applied to chronic exposure or consumption.

In view of the close chemical relationships and the similar toxicological mechanisms manifested by toxaphene and other chlorinated hydrocarbons, a limited amount of information is provided in this document on these additional compounds to provide comparative information on relative effects or dose levels causing a specific effect.

ACKNOWLEDGEMENTS

Members of the Provincial and Federal Departments of Environment, Health and Welfare and Fisheries were written and informed of the project and its objectives and asked to provide any relevant data which they could make available. Various Federal and Provincial authorities, such as Statistics Canada, the Ministry of Trade and Commerce, etc., were contacted for statistics related to manufacturing and use of toxaphene. Several individuals in various U.S.A. agencies, such as EPA, NIOSH and FDA, were also contacted and requested to provide relevant information. Much useful data were obtained in this fashion and included individual communications and opinions, as well as reviews and criteria documents. The response to our information requests were excellent and unquestionably yielded information which would not have surfaced by standard literature search methods. The cooperation of all of those individuals who took time to respond is gratefully acknowledged.

INDEX

PAGE

1. OVERVIEW - VUE D'ENSEMBLE 1

2. CHEMISTRY AND ANALYTICAL METHODS 17
  - 2.1 Nomenclature 17
  - 2.2 Synthesis 17
  - 2.3 Physical And Chemical Properties 19
  - 2.4 Nature And Content Of Commonly Associated Impurities 21
  - 2.5 Analytical Methodology 21
    - 2.5.1 General Methods 21
    - 2.5.2 Specific Methods 25
      - 2.5.2.1 Water 25
      - 2.5.2.2 Food 29
      - 2.5.2.3 Air 31
      - 2.5.2.4 Aquatic Biota 32
      - 2.5.2.5 Soil And Sediment 36
  - 2.6 Uses 38

3. SOURCES OF ENVIRONMENTAL POLLUTION 40
  - 3.1 Natural Occurence 40
  - 3.2 Synthetic Production 41
  - 3.3 Utilization Patterns 41
    - 3.3.1 Extent of Use 41
      - 3.3.1.1 Industry 41
      - 3.3.1.2 By Research Establishments 42
      - 3.3.1.3 By The General Public 42
    - 3.3.2 Industrial Release To Air And Water 43
      - 3.3.2.1 Intentional 43
      - 3.3.2.2 Accidental 43
      - 3.3.2.3 Waste Disposal 43
  - 3.4 Other Means Of Production 44
  - 3.5 Availability And Effectiveness Of Control Technology 45

4. ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION 46
  - 4.1 Routes And Mechanisms Of Transport Into The Various Environmental Media 46
    - 4.1.1 Water 46
    - 4.1.2 Air 50
    - 4.1.3 Soil 50
  - 4.2 Transfer Between Media And Modes Of Degradation 50
    - 4.2.1 General Movement 50
    - 4.2.2 Bioconcentration 52

INDEX (Continued)

PAGE

4.2.3 Interaction With Other Physical, Chemical And Biological Factors 55

4.3 Persistence Of Toxaphene And Its Degradation Products 56

5. ENVIRONMENTAL EXPOSURE LEVELS 58

5.1 Environmental Levels 58

5.1.1 Water 58
5.1.2 Food 60
5.1.3 Air 64
5.1.4 Soil And Sediment 67
5.1.5 Aquatic Biota 69

6. EXPERIMENTAL TOXICOLOGY 70

6.1 Metabolism 70
6.2 Acute Oral And Parenteral Toxicity 74

6.2.1 Mammals 74

6.2.1.1 Dogs 74
6.2.1.2 Rodents 78
6.2.1.3 Others 80

6.2.2 Birds 80
6.2.3 Insects 82
6.2.4 Fish 92
6.2.5 Plankton And Algae 100
6.2.6 Plants 101

6.3 Sub-Acute Toxicity 102

6.3.1 Mammals 102
6.3.2 Birds 104
6.3.3 Fish 104

6.4 Chronic Toxicity 107

6.4.1 Mammals 107

6.4.1.1 Rodents 107
6.4.1.2 Dogs 109
6.4.1.3 Monkeys 113

6.4.2 Fish 113

6.5 Dermal Toxicity 114
6.6 Inhalation Toxicity 116
6.7 Biochemical Effects Of Toxaphene 116

6.7.1 Mammalian Systems 116

6.7.1.1 Rats 116
6.7.1.2 Cattle 118

6.7.2 Aquatic Systems 119

INDEX (Continued)

PAGE

6.8 Carcinogenicity 120
6.9 Mutagenicity 121
6.10 Reproductive Studies 121
  6.10.1 Rodents 121
  6.10.2 Birds 122
6.11 Teratogenesis 123
6.12 Interactions And Synergistic Effects 124
  6.12.1 Insects 124
  6.12.2 Rodents 125
  6.12.3 Fish 126

7. HUMAN HEALTH EFFECTS 126

7.1 Metabolism 127
7.2 Acute Toxicity 128
7.3 Clinical Effects And Treatment Of Overdose 134
7.4 Epidemiology 136

8. EXPOSURE, DOSE ASPECTS 136

8.1 Dose-Effect And Dose-Response Relationships 136
  8.1.1 Body Weight 137
  8.1.2 Effects On Liver 137
  8.1.3 Effects On The CNS 137
  8.1.4 Effects On The Kidneys 137
  8.1.5 Effects On Biochemical Parameters 138
  8.1.6 Reproduction And Teratogenesis 138
8.2 Calculated Or Estimated Recommended, Acceptable Or Tolerable Doses From Available Data 138
8.3 Current Exposure From Food, Air And Water 143
8.4 Special Risk Groups 143
8.5 Published Figures From Other Organizations 143

9. RECOMMENDATIONS FOR FURTHER RESEARCH 146

1. OVERVIEW

Toxaphene is a major chlorinated insecticide used in the United States and is employed primarily in the control of cotton pests. It is no longer employed for general agricultural purposes in Canada, but is registered for use. Its use in Canada appears to be limited to special instances and it appears that toxaphene is not readily available to the farming community in Canada. In the U.S.A. toxaphene is applied to crops mainly by aerial spraying. In addition, the insecticide has been used to kill "rough" fish in lakes and water systems. It was estimated that virtually the entire U.S. annual production of over 50 million pounds was released into the environment by way of these two routes. In Canada, toxaphene would not be released to the atmosphere, except in the rare instances when it is used for special purposes.

Toxaphene is referred to by various other chemical names throughout the world, with the most common alternate nomenclature being camphechlor. It is produced by the chlorination of camphene resulting in a mixture of chlorinated hydrocarbons containing 67 to 69% by weight of chlorine, and corresponding to an overall empirical formula of $C_{10}H_{10}Cl_8$. Technical toxaphene is a complex mixture and it has been estimated that it contains at least 177 different polychlorinated derivatives.

Gas chromatographic-mass spectrometry data indicate that a significant fraction of commercial toxaphene is made up of isomeric hepta-, octa- and nona- chlorobornanes. To date, only about 8 components of toxaphene have been isolated, with three of the structures having been definitively characterized and the structure of three additional compounds partially determined. Two of the known components have been named toxicants A and B, and these, together with various crude fractions, as well as technical toxaphene, have been investigated for their relative toxicity.

The most acceptable method for the chemical analysis of toxaphene, particularly at the low levels found in water and food, requires rigorous clean-up procedures. Generally, extraction with organic solvents such as petroleum ether or diethyl ether are employed, together with acid treatment for the removal of interfering substances and other chlorinated pesticides, followed by concentration of the extracts, and analysis using gas chromatography with electron-capture detection. Columns of SE-30 and QF-1 or DC-200 are commonly used for separation of toxaphene.

The gas chromatographic assay of toxaphene gives a scan consisting of about 12 distinct major peaks, which may be quantitated by measuring the area under the total "fingerprint" or under three of the most prominent peaks. Because some of the components elute slowly, confusion or interference from DDT or PCB's may be a problem and care must be taken that these compounds do not result in erroneous quantitation of toxaphene. In general, the methods for the analysis of toxaphene in water are applicable to samples containing less than 5 ug/l (as low as 1 ug/l).

Toxaphene, like other chlorinated hydrocarbons, is bioaccumulated or concentrated by various non-target organisms. It has been shown that molluscs, fish, algae and insects bioconcentrate toxaphene 20,000 fold or greater from an aqueous media. In animals, toxaphene accumulates primarily in fat, where it has been found to reach a finite storage level, after which no further increase in concentration occurs. When the source of toxaphene is removed, the residue is rapidly reduced and, in this regard, the elimination of toxaphene is more rapid than most other chlorinated hydrocarbons.

The half-residence time of toxaphene in soil depends to a large extent on the type of soil, and may range from 100 days to as long as 6 to 12 years. Experimental data indicate that toxaphene is detoxified more rapidly in hard water than in soft water, and in waters with high oxygen concentration and temperature.

No reports of water contamination by toxaphene have been reported in Canada, including the data derived from the Ontario Water Surveys carried out in 1974-1976. Toxaphene has been identified in drinking water samples in the United States, particularly in the southern states where the pesticide is used extensively on cotton and soy bean crops; but even there, concentrations reported have generally been less than 1 ug/l.

It is likely that there is a low incidence of toxaphene in the Canadian food supply. The Total Diet Pesticide Residue Program carried out by Health and Welfare Canada from 1969 to 1973 found no evidence of detectable levels of toxaphene in any foodstuffs. During the 1974-1975 survey, the incidence of toxaphene in food samples was reported as 0.6%; but no quantitative data, or indication of the type of food in which toxaphene was found, was given.

The chemical complexity of toxaphene makes the study of the metabolism of the compound difficult. Toxaphene is believed to be slowly detoxified in the liver and excreted as the ethereal sulfate and glucuronic acid conjugates in the urine. After administering ^{36}Cl and ^{14}C labelled compounds to rats, approximately 50% of the dose was found to be excreted as chloride ion in the urine. Similar findings were found for toxicants A and B, as well as for other fractions, each of which contained 25 to 50 individual components. In each instance about 50% of the carbon-chlorine bonds were metabolically active. Of the total radioactive compound administered, 60% of the dose was recovered, with 35% being in the feces and 25% in the urine. The expiration of $^{14}CO_2$ accounted for only about 1% of the administered dose. The major route of metabolism of toxaphene appeared to be dechlorination and/or dehydrochlorination.

After an oral dose of the pesticide, the material distributes throughout most body tissues, with the highest accumulation being in fat, kidney, and liver. An oral dose of 8.5 mg/kg to rats resulted 14 days later in fat containing 0.52, liver 0.12, kidney 0.17, blood 0.14 and other tissues 0.02 to 0.09 mg/kg.

The oral toxicity of toxaphene varied with the vehicle used, and absorption increased with the use of absorbable oils, such as vegetable oils, and decreased with non-absorbed solvents, such as kerosene. One of the earliest signs of acute toxicity in animals was convulsions. Toxaphene at 15 mg/kg in corn oil resulted in periodic convulsions and death in dogs. The rat oral LD_{50} varied from 45 mg/kg to 350 mg/kg, depending on the vehicle used and other study parameters. The intravenous LD_{50} in rats has been recorded at about 13 mg/kg. For example, toxicant A was reported as being 10-15 times as toxic as toxaphene itself.

Large differences in the toxicity of various fractions of toxaphene have been noted.

Birds and small laboratory animals have approximately equal sensitivity to toxaphene. In this latter respect, toxaphene differs from other chlorinated pesticides.

Toxaphene has been found to be more toxic to fish than other chlorinated pesticides, and it has been used as a piscicide to eradicate fish from water systems. It is an extremely potent fish poison and a concentration in water of 0.02 to 0.1 mg/l (ppm) was effective as a piscicide. Toxaphene was reported as being relatively persistent in water and it may be 1 to 5 years before restocking with fish can occur. Toxaphene was more toxic in hard than in soft water and its toxicity increased with increasing water temperature. The acute toxicity, as measured by the 96-hour TL_{50}, varied with the species of fish tested, with bass having a TL_{50} of 1 ug/l and goldfish 14 ug/l.

In general, toxaphene was not considered to be phytotoxic.

Little subacute toxicity data were available in the literature, but cows receiving diets containing up to 140 mg/kg showed no signs of toxicity after 8 weeks of feeding. Dogs given daily doses of 5 mg/kg body weight survived for at least 4 years.

The findings from chronic feeding studies in animals have given variable indications as to the toxicity of toxaphene.

Liver cell abnormalities were observed in rats receiving 50 mg/kg in their diet for 6 to 9 months (estimated intake of 2.5 mg/kg/day). Others found that 100 mg/kg in the diet (2.8 mg/kg/day) had no effect, but at 1,000 mg/kg after 7.5 to 10 months, convulsions occurred in a few animals and liver changes were apparent on histopathological examination.

In lifetime feeding studies, rats fed 100 or 400 mg/kg in the diet had centrilobular hepatic cell enlargement. Minimal effects were seen in rats administered a diet containing 25 mg/kg (1.25-1.40 [illegible]kg/day). In general, the effects of chronic administration of toxaphene were similar to other chlorinated hydrocarbons, with the main response being centrilobular liver cell enlargement, involving structural changes in the mitochondria and endoplasmic reticulum. Kidney cell degeneration also occurred.

Dogs given 50 mg/kg in the diet (3.12-6.56 mg/kg body weight/day) on initial study were reported as having moderate liver degeneration, but reexamination of the tissues failed to confirm this finding. Dogs receiving 10 mg/kg (0.6-1.47 mg/kg/day) had no observable effects.

No changes in body weights or organ to body weight ratios were seen in dogs receiving 20 mg/kg or less in their diet (estimated to be equivalent to about 0.3-0.7 mg/kg body weight/day), and, in addition, no histopathological changes in liver or other tissues were observed after two years of pesticide administration.

It was reported in 1965 that the dog was the most sensitive species, with 1.7 mg/kg/day causing minimal or no long-term effects. However, the findings of Fitzhugh and Nelson, that the no-effect dose in the rat was 1.25 mg/kg/day, indicate the rat to have equal or greater sensitivity to the toxic effects of toxaphene.

Monkeys fed 0.6-0.78 mg/kg/day were normal after administration 6 days a week for 2 years. In this study no dose level was used which caused an observable effect and, therefore, no minimal effective dose was determined.

Systemic effects of toxaphene may be induced by absorption through the skin, when applied in dry form or in solution, with absorption through abraded skin surfaces being the most rapid. The rate and extent of the cutaneous absorption of toxaphene from powders was variable and depended on the concentration and extent of exposure, as well as the presence of wetting agents (as used in wettable powders) and the susceptibility of the animal species. High concentrations of dust (50%) did not elicit symptoms in dogs, although lesser concentrations were lethal to rabbits. Oil and emulsion formulations of the chemical were readily absorbed through the skin and were the most dangerous.

Toxaphene caused increased liver microsomal enzyme activity. Diets containing 5 mg/kg or more of toxaphene fed to young rats (less than 1 mg/kg body-weight/day), caused increased microsomal enzyme activity, as measured by the metabolism of drugs and hormones. A dose of 2.4 mg/kg/day caused a decrease in liver LDH activity. In fish, a concentration of toxaphene of 0.01 mg/l in the surrounding water resulted in structural changes in liver mitochondria and rough endoplasmic reticulum.

In the United States, the National Cancer Institute and the Hercules Chemical Corporation both are investigating the carcinogenic potential of toxaphene. Preliminary reports from the NCI study suggested that it was carcinogenic to mice and rats, but no information as to the dose levels used have been published. The Hercules study is scheduled to be completed in 1978.

In reproductive and teratogenic studies, 25 mg/kg in the diet of rats had no effect, whereas a 100 mg/kg diet resulted in liver enlargement, but no changes in reproductive capabilities. Similar findings were reported with mice at a dietary level of 25 mg/kg.

It appears that 25 mg/kg (about 1.25-1.40 mg/kg/day) in the diet approximates the no-effect level for that species. Therefore, the rat and dog would appear to have similar propensities to the toxic effects of toxaphene.

Gross pathologic findings in animals receiving toxaphene were seen mainly as degenerative changes in the renal tubules and liver parenchyma. The latter were characterized by centrilobular hypertrophy of the hepatic cells, with eventual necrosis and reparative hypertrophy. Other organs of the body were relatively unaffected by the chronic administration of toxaphene, especially at the lower dose levels which caused minimal liver damage.

Although little experimental evidence was available on the absorption, distribution, metabolism and excretion of toxaphene by man, most toxicologists appeared to assume it was absorbed via the intestines, lungs and skin in as variable a manner as has been reported for animals. In addition, it is assumed that toxaphene is distributed throughout the body fat depots, although to a lesser extent than other chlorinated pesticides or organophosphate compounds.

The acute lethal oral dose for an adult has been estimated to be 2 to 7 grams, which suggests that toxaphene is about 4 times as toxic as DDT.

Although toxaphene was used extensively for agricultural and public health programs in Alberta during the 1960's, analysis of tissues, in 1967-1968, from autopsy specimens of 50 cases failed to show any detectable levels of the pesticide in any of the tissues examined. No other epidemiological data of value in setting water standards were reviewed.

Because of a paucity of information, estimates of the maximum allowable daily intake of toxaphene by man could not be determined using human toxicological data.

In a discussion of the extrapolation of animal data in predicting safety in man, the author of the present document calculated an ADI of 0.00125 mg/kg/day. This was derived using the minimal effect dose for the rat of 1.25 mg/kg/day and applying a 1,000-fold "safety" factor.

In the U.S.A., the Environmental Protection Agency recommended a drinking water standard of 24 ug/l based on health effects, but organoleptic considerations lowered the value to 5 ug/l. In order to protect aquatic organisms, however, a level of 0.005 ug/l was suggested.

Recommendations for future research are presented in section 9 of the document.

1. VUE D'ENSEMBLE

Le toxaphène est un important insecticide chloré utilisé aux Etats-Unis; il sert principalement à lutter contre les parasites du coton. Au Canada, le toxaphène n'est plus employé à des fins agricoles générales, mais demeure néanmoins enregistré. Son utilisation semble s'y être limitée à des applications spéciales; il semble d'ailleurs que la communauté agricole du Canada n'ait pas facilement accès au toxaphène. Aux États-Unis, on utilise surtout l'avion pour pulvériser le toxaphène sur les cultures. Cet insecticide a en outre été utilisé pour éliminer des lacs et des rivières certains poissons sans valeur commerciale. On a estimé que la presque totalité de la production annuelle de toxaphène aux États-Unis (plus de 50 millions de tonnes) a été déversée dans l'environnement par l'un ou l'autre de ces moyens. Au Canada, le toxaphène n'est utilisé que dans de rares cas spéciaux.

Le toxaphène a reçu une foule d'autres appellations chimiques dans le monde, la plus répandue étant probablement celle de chlorocamphène. On l'obtient par chloration du camphène; le mélange d'hydrocarbures chlorés qui en résulte contient, en poids, 67 à 69% de chlore et peut être représenté par la formule empirique globale de $C_{10}H_{10}Cl_8$. Le toxaphène technique est un mélange complexe; selon les estimations, il contient au moins 177 dérivés polychlorés.

Les données recueillies en spectrométrie de masse et en chromatographie en phase gazeuse révèlent qu'une fraction importante du toxaphène commercial est composée d'hepta-, octa- et nona- chlorobornanes isomères. Jusqu'à ce jour, on n'a pu isoler qu'environ 8 composants du toxaphène - trois de ces structures ont été définies avec précision et trois autres le sont de façon partielle. Deux des composants connus ont été baptisés les toxiques A et B; les tests de toxicité relative ont principalement porté sur les toxiques A et B, diverses fractions brutes et le toxaphène technique.

L'analyse chimique du toxaphène doit, pour être faite dans les meilleures conditions, être précédée d'une purification soignée, particulièrement s'il est contenu dans de l'eau ou des aliments, car les quantités d'agents toxiques y sont peu élevées. De façon générale, l'extraction se fait à l'aide de solvants organiques (éther de pétrole, éther diéthylique) et elle est suivie d'un traitement à l'acide destiné à supprimer les éléments étranger et les autres pesticides chlorés; l'échantillon est alors concentré, puis analysé par chromatographie en phase gazeuse avec détection à capture d'électrons. La séparation du toxaphène se fait généralement sur des collonnes de SE-30 et QF-1 ou DC-200.

La chromatographie en phase gazeuse nous donne une image qui comporte 12 pics distincts qu'on peut quantifier en mesurant la superficie totale sous le tracé ou sous les trois pics les plus importants. Comme certains des composants ne s'élèvent que lentement, il se peut qu'on les confonde avec le DDT ou les PCB; il faut donc prendre grand soin de ne pas assimiler ces éléments à du toxaphène, car cela fausserait les résultats. De façon générale, les méthodes d'analyse du toxaphène dans l'eau s'appliquent aux échantillons contenant moins de 5µg/l (et jusqu'à 1 µg/l).

Tout comme les autres hydrocarbures chlorés, le toxaphène s'accumule ou se concentre dans divers organismes non spécifiques. On a constaté que les mollusques, les poissons, les algues et les insectes concentraient au moins 20 000 fois le toxaphène d'un milieu aqueux. Chez les animaux, le toxaphène s'accumule principalement dans les tissus adipeux; on a observé que cette accumulation atteignait un niveau donné et que, par la suite, elle demeurait stable. Lorsque la source de toxaphène disparaît, les quantités emmagasinées diminuent rapidement; à cet égard, le toxaphène s'élimine plus rapidement que la plupart des autres hydrocarbures chlorés.

La période de demi-vie du toxaphène dans le sol dépend dans une large mesure de la nature du sol et peut se situer entre 100 jours et 6 à 12 ans. Selon les données expérimentales, le toxaphène se détoxifie plus rapidement dans de l'eau dure que dans de l'eau douce et dans de l'eau contenant une forte concentration d'oxigène et à température élevée.

Jusqu'ici, les analyses - et notamment les enquêtes sur la qualité de l'eau faites par l'Ontario de 1974 à 1976 - n'ont pas mis en évidence la présence de toxaphène dans l'eau du Canada. On a néanmoins décelé la présence de toxaphène dans des échantillons d'eau potable prélevés aux États-Unis, particulièrement dans les États du sud, où le pesticide est utilisé en grande quantité sur les cultures de coton et de soya. Même dans ces cas, toutefois, les concentrations étaient généralement inférieures à 1µg/l.

Il est probable qu'il y a peu de toxaphène dans les aliments offerts à la consommation au Canada. Les résultats du Programme de mesure des résidus de pesticides dans le régime total, du ministère de la Santé nationale et du Bien-être social, révèlent que, de 1969 à 1973, on n'a pas trouvé de traces décelables de toxaphène dans les produits alimentaires. Au cours de l'enquête de 1974-1975, par contre, on a décelé la présence de toxaphène dans 0.6% des échantillons d'aliments; le ministère n'a toutefois pas publié de renseignements sur les quantités trouvées, ni sur la nature des aliments qui contenaient du toxaphène.

Le toxaphène étant chimiquement complexe, il est difficile d'en étudier le métabolisme. On croit qu'il perd lentement ses propriétés toxiques dans le foie et qu'il est excrété dans l'urine sous forme de conjugés d'acide glucuronique et d'éther-sulfate. Après avoir administré des produits marqués au ^{36}Cl et au ^{14}C à des rats, on a constaté qu'environ 50% de la dose avait été excrétée dans l'urine sous la forme d'ions chlorures. Les mêmes observations ont été faites pour les agents toxiques A et B de même que pour d'autres fractions (certaines d'entre elles contenant de 25 à 50 composants). Dans chaque cas, près de 50% des liaisons carbone-chlore étainet métaboliquement actives. 60% de la dose radioactive totale a été récupérée (35% dans les fèces et 25% dans l'urine). L'expiration de $^{14}CO_2$ ne représentait qu'environ 15 de la dose administrée. Le toxaphène est donc métabolisé principalement par voie de déchloration et (ou) de déshydrochloration.

Pris par voie orale, le toxaphène se répartit dans la plupart des tissus du corps et se concentre principalement dans les tissus adipeux, les reins et le foie. On a administré à des rats une dose de 8.5 mg/kg par voie orale; 14 jours plus tard, les tissus adipeux contenaient 0.52 mg/kg de toxaphène, le foie, 0.12, les reins, 0.17, le sang, 0.14 et les autres tissus 0.02 à 0.09.

La toxicité orale du toxaphène varie en fonction du vecteur utilisé; l'absorption, pour sa part, augmente si l'on utilise des huiles absorbales telles que les huiles végétales, mais diminue lorsque le vecteur utilisé est un solvant non absorbé tel que le kérosène. Chez les animaux, les convulsions constituent l'un des premiers signes de toxicité aigue. Une dose de 15 mg/kg administrée dans de l'huile d'olive à des chiens a entraîné des convulsions périodiques et la mort. Chez le rat, la dose orale léthale (DL_{50}) se situe entre 45mg/kg et 350 mg/kg, compte tenu du vecteur utilisé et d'autres paramètres; la dose léthale intraveineuse (DL_{50}) est d'environ 13 mg/kg. On a remarqué que l'agent toxique A était de 10 à 15 fois plus toxique que le toxaphène lui-même.

Il existe des écarts marqués entre la toxicité des diverses fractions de toxaphène.

Les oiseaux et les petits animaux de laboratoire ont à peu près la même sensibilité au toxaphène qui, à cet égard, diffère des autres pesticides chlorés.

On a découvert que les poissons sont plus sensibles au toxaphène qu'aux autres pesticides chlorés; c'est pourquoi on l'utilise comme piscicide. Le toxaphène est un piscicide très puissant en concentration dans l'eau de 0.02 à 0.1 mg/l. Le toxaphène est relativement persistant dans l'eau; il peut en effet y demeurer actif de 1 à 5 ans. Il est plus toxique dans l'eau dure que dans l'eau douce et sa toxicité augmente si la température de l'eau s'accroît. Mesurée en fonction d'une LT_{50} de 96 heures, la toxicité aigue varie d'une espèce de poisson à l'autre (achigan: 1 µg/l, cyprin doré: 14 µg/l.

Le toxaphène n'est pas considéré phytotoxique.

Il existe de façon générale peu de données sur la toxicité subaiguë du toxaphène; toutefois, on a constaté que des vaches à qui on avait administré jusqu'à 140 mg/kg de toxaphène ne manifestaient pas de signes d'intoxication 8 semaines après l'administration du produit. Des chiens à qui on avait administré tous les jours une dose de 5 mg/kg ont survécu pendant au moins 4 ans.

Les résultats d'études d'alimentation chronique de certains animaux nous donnent des indications variables quant à la toxicité du toxaphène.

On a observé des anomalies dans les cellules du foie de rats à qui on avait administré dans leur alimentation 50 mg/kg de toxaphène pendant 6 à 9 mois (apport estimatif de 2.5 mg/kg par jour). D'autres chercheurs ont constaté qu'une addition de 100 mg/kg dans le régime (2.8 mg/kg par jour) n'avait pas d'effet, mais que, après une absorption de 1000 mg/kg pendant 7.5 à 10 mois, on observait des convulsions chez certains spécimens et que les examens histopathologiques révélaient des transformations du foie.

Dans les études d'alimentation à vie, on a observé un gonflement des cellules hépatiques centrilobulaires chez les rats à qui on en avait administré de 100 à 400 mg/kg dans le régime. Les effets minimaux se voient chez les rats dont l'alimentation contenait 25 mg/kg de toxaphène (1.25 à 1.40 mg/kg par jour). De façon générale, les effets de l'administration chronique de toxaphène étaient analogues à ceux des autres hydrocarbures chlorés, le principal d'entre eux étant le gonglement des cellules hépatiques centrilobulaires et les modifications structurelles des mitochondries et du réticulum endoplasmique qui s'ensuivent. On a également observé des cas de dégénérescence des cellules des reins.

Les chiens à qui on a ajouté 50 mg/kg de toxaphène dans l'alimentation (3.12 à 6.56 mg/kg par jour) lors des premières études ont subi une légère dégénérescence du foie; toutefois, cette observation n'a pas été confirmée par un réexamen des tissus. Les chiens ayant reçu une dose de 10 mg/kg (0.6 à 1.47 mg/kg par jour) n'ont pas subi d'effets observables.

On n'a pas observé de modifications du poids du corps ou du rapport poids des organes/poids du corps chez les chiens à qui on a ajouté dans leur alimentation 20 mg/kg ou moins de toxaphène (apport estimatif d'environ 0.3 à 0.7 mg/kg par jour); de plus, le foie et les autres tissus ne semblent pas avoir subi de modifications histopathologiques en deux ans d'administration du pesticide.

On a déclaré en 1965 que le chien était l'espèce la plus sensible au toxaphène, seule une dose de 1.7 mg/kg par jour ne causant pas ou pratiquement pas d'effets à long terme. Toutefois, les observations de Fitzhugh et Nelson semblent indiquer que le rat a une sensibilité égale ou supérieure aux effets toxiques du toxaphène, la dose sans effet, dans ce cas, étant de 1.25 mg/kg par jour.

Les singes à qui on a administré 0.6 à 0.78 mg/kg par jour de toxaphène 6 jours par semaine pendant deux ans ne présentaient pas d'anomalies. Comme on n'a pas utilisé de dose pouvant provoquer des effets mesurables dans cette étude, la dose minimale effective n'a pas pu être déterminée.

Les effets systématiques du toxaphène peuvent être provoqués par l'absorption cutanée, lorsque le pesticide est appliqué sous forme sèche ou en solution; l'absorption se fait le plus rapidement si la surface de la peau est éraflée. Le rythme et l'importance de l'absorption cutanée du toxaphène en poudre dépend de sa concentration, du degré d'exposition, de la présence d'agents mouillants et de la susceptibilité de l'expèce animale. Les fortes concentrations de poussières (50%) ne provoquent pas de symptômes chez les chiens; par contre, des concentrations inférieures sont fatales pour le lapin. Associé à de l'huile ou présenté en émulsion, le toxaphène peut être facilement absorbé par la peau; c'est là qu'il devient le plus dangereux.

Le toxaphène provoque un accroissement de l'activité des enzymes des microsomes du foie. Si l'on ajoute à l'alimentation des rats 5mg/kg et plus de toxaphène (moins de lmg/kg par jour), on provoque, comme le révèle une étude du métabolisme et des hormones, un accroissement de l'activité des enzymes des microsomes. Une dose de 2.4 mg/kg par jour provoque une diminution de l'activité de la LDH dans le foie. Chez le poisson, on observe des transformation structurelles des mitochondries et du réticulum endoplasmique du foie lorsque la concentration du toxaphène dans l'eau environnante est de 0.1 mg/l.

Aux États-Unis, le National Cancer Institute et la Hercules Chemical Corporation ont tous deux étudié le pouvoir carcinogène du toxaphène. Les résultats préliminaires obtenus par le NCI laissent entrevoir que le toxaphène peut être carcinogène dans le cas des souris et des rats; le NCI n'a toutefois pas publié de renseignements sur les dosesutilisées. L'étude de la Hercules devrait être terminée en 1978.

Dans les travaux sur la reproduction et les études tératogéniques, on a montré qu'une addition de 25 mg/kg de toxaphène dans l'alimentation des rats ne provoquait aucun effet, alors qu'une addition de 100 mg/kg provoquait une augmentation du volume du foie, mais n'agissait pas sur les capacités de reproduction. On a fait des constatations analogues en ajoutant une dose de 25 mg/kg de toxaphène au régime alimentaire des souris.

Il semble qu'une dose de 25 mg/kg dans l'alimentation (soit environ 1.25 à 1.40 mg/kg par jour) ne provoque *aucun effet chez cette espèce. Le rat et le chien* semblent donc réagir de la même façon aux effets toxiques du toxaphène.

Les principales anomalies pathologiques observées chez les animaux à qui on a administré du toxaphène ont principalement pris la forme d'une dégénérescence des tubules rénaux et du parenchyme du foie. Dans ce dernier cas, il y eut hypertrophie centrilobulaire des cellules hépatiques, nécrose et hypertrophie de reconstitution. Les autres organes du corps ont été peu touchés par l'administration chronique de toxaphène, particulièrement lorsque les doses ont été voisines du niveau causant les effets minimaux au foie.

Bien qu'il y ait peu de données expérimentales sur l'absorption, la distribution, le métabolisme et l'excrétion du toxaphène chez l'homme, la plupart des toxicologues semblent supposer que son absorption se fait par les intestins, les poumons et la peau, tout comme dans le cas des animaux. De plus, on a supposé que le toxaphène se répartissait dans tous les tissus adipeux du corps, mais à un moindre degré que les autres pesticides chlorés ou que les composés organophosphatés.

Selon les estimations, la dose orale léthale aiguë pour l'adulte est de 2 à 7 grammes, ce qui veut dire que le toxaphène est environ 4 foie plus toxique que le DDT.

Bien que le toxaphène ait été largement utilisé à des fins agricoles et dans le cadre de programmes d'hygiène publique en Alberta au cours des années 1960, une analyse de tissus faite en 1967-1968 dans le cadre de l'autopsie de 50 corps n'a pas permis de déterminer la présence de niveaux décelables de toxaphène dans les tissus examinés. Nous n'avons pas analysé d'autres données épidémiologiques intéressantes.

En raison de la rareté des renseignements, il n'a pas été possible de déterminer la quantité maximale de toxaphène que l'homme peut absorber quotidiennement à partir de chiffres sur les effets toxiques du toxaphène sur l'homme.

S'aidant d'extrapolations pour déterminer la dose quotidienne admissible chez l'homme, l'auteur de cette étude a établi qu'elle devrait se situer à 0.00125 mg/kg par jour. Ce chiffre a été obtenu en utilisant la dose minimale provoquant un effet chez le rat (1.25 mg/kg par jour) et en lui appliquant un facteur de "sécurité de 1000.

Aux États-Unis, la Environmental Protection Agency a fixé une norme de 24 µg/l dans le cas de l'eau potable en s'appuyant sur les effets sur la santé; toutefois, des considérations d'ordre organoleptique ont fait passer ce chiffre à 5 µg/l. Afin de protéger les organismes aquatiques, cependant, on a proposé un niveau de 0.005 µg/l.

On trouvera dans la section 9 de cette étude des recommandations quant à l'orientation que pourraient prendre les travaux de recherche futurs sur le toxaphène.

2. CHEMISTRY AND ANALYTICAL METHODS

2.1 Nomenclature:

Toxaphene or camphechlor (ISO approved name) is variously called chlorinated camphene, octachlorocamphene, polychlorocamphene (U.S.S.R.), phenacide, phenatox, alltox, geniphene, phenphene or toxakil. Toxaphene is a registered trade mark in some countries and is a common name in others (1, 2, 3).

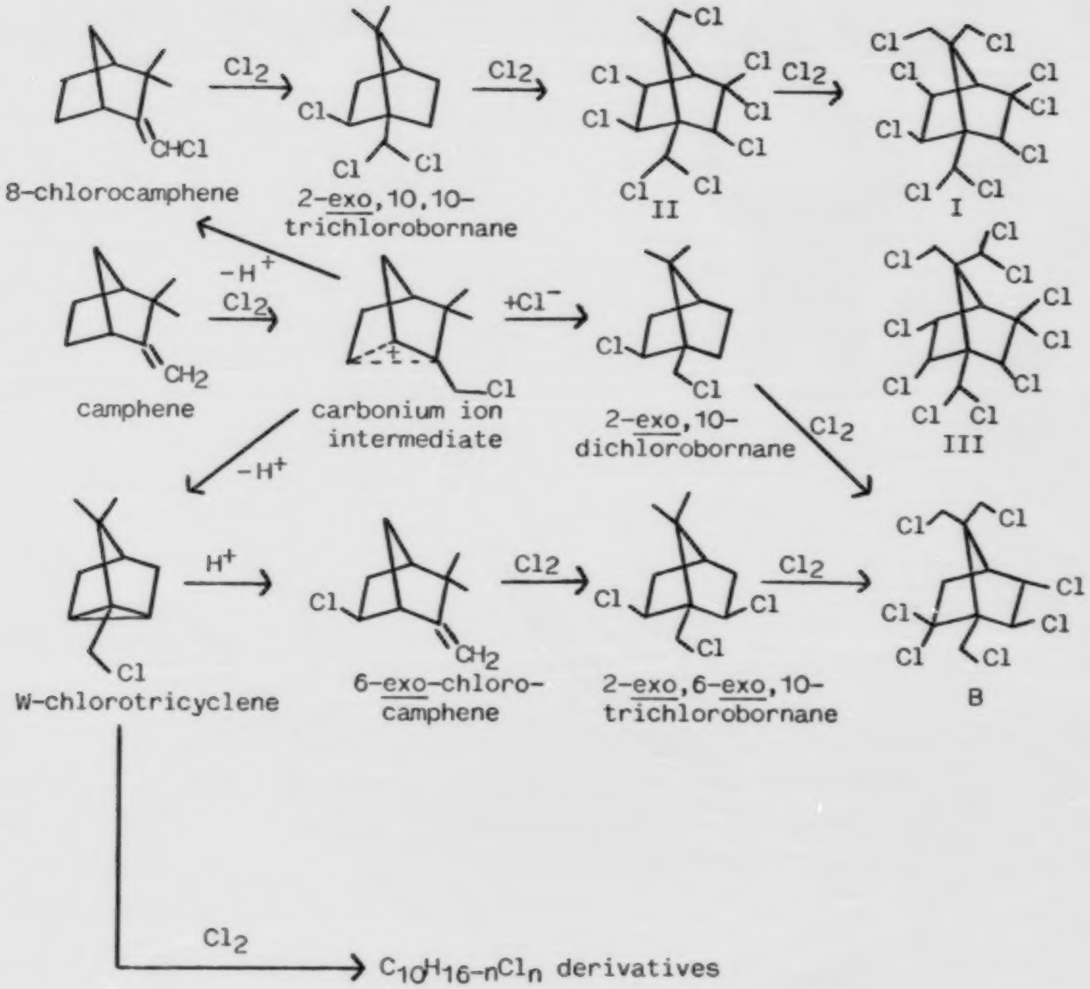
2.2 Methods of Synthesis:

Toxaphene is an insecticidal composition produced by the chlorination of at least one of the following: camphene, camphene hydrochloride, or isocamphene. The reaction is carried out in carbon tetrachloride using chlorine gas, catalysts and ultraviolet light at an elevated temperature. The resulting mixture (toxaphene) has a chlorine content of 67 - 69% by weight, which corresponds to an overall empirical formula of $C_{10}H_{10}Cl_8$ (4, 5). The number of individual chemicals comprising toxaphene is estimated to be about 177 (5, 6, 7).

A diagrammatic representation of the early intermediates formed during toxaphene synthesis was given by Casida et al (5) and is shown in Figure 1. Further work has indicated that, in addition to the presence of polychlorobornanes in technical toxaphene, polychlorodihydrocamphenes may also be present (8, 9). Landrum et al (9) postulated an additional route via which toxaphene was formed during the chlorination process (Fig.2) and commented that it was entirely probable that polychlorotricyclenes may also be present in technical toxaphene.

Benson (10) stated that when one examined its synthesis by chlorination of camphene, using 1:1 molar ratios of chlorine and camphene it was clear why so many compounds existed in technical toxaphene. The reaction involves rearrangement, elimination and addition. Potassium hydroxide, present during the synthetic reaction, causes varying degrees of dechlorination to occur, which also results in a multitude of compounds.

FIGURE 1. TOXAPHENE SYNTHETIC PATHWAY

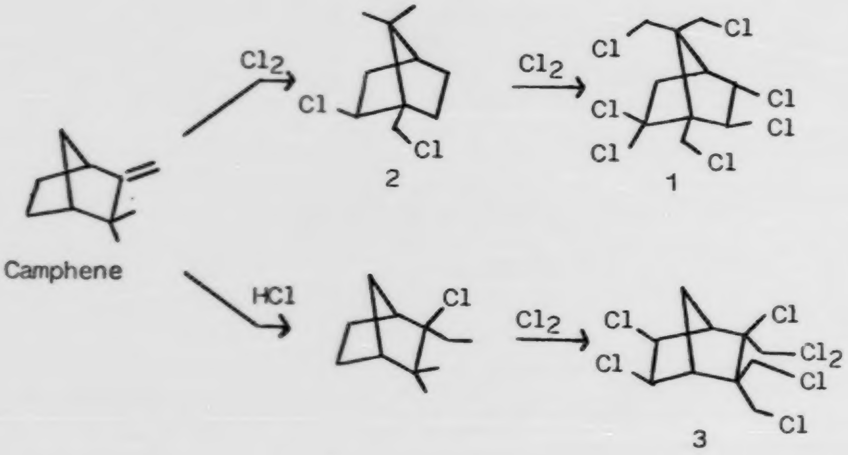


Chlorination of camphene in carbon tetrachloride using ultraviolet light and certain other catalysts showing early intermediates, one identified Toxaphene constituent (B), and structures proposed for three other polychlorobornane Toxaphene components (I, II and III).

(From reference 5)

FIGURE 2. TOXAPHENE SYNTHESIS LEADING TO THE FORMATION OF CHLORODIHYDROCAMPHENE

(From Reference 9)



1 = 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane

2 = *exo*-2,10-dichlorobornane

3 = 2,5,6-*exo*,8,8,9,10-chlorodihydrocamphene

2.3 Physical And Chemical Properties (2, 3, 11, 12, 13)

Technical toxaphene is an amber, waxy solid with a melting range of from 70 to 95°C. It has a mild terpene or pine-like odor. Toxaphene dechlorinates on heating above 120°C or when exposed to ultraviolet light or strong sunlight. Dechlorination is accelerated in alkaline media and catalyzed by iron. Toxaphene is non-corrosive in the absence of moisture and is stable in acid. Toxaphene is practically insoluble (about 3 ppm) in water at 20°C, sparingly soluble in alcohols (10 - 18 g/100 ml) at 270°C, but is highly soluble in turpentine, kerosene and fuel oil (250 - 400 g/100 ml). It is freely soluble in acetone, benzene, carbon tetrachloride, ethylene dichloride, toluene and xylene.

Empirical formula and molecular weight: $C_{10}H_{10}Cl_8$, MW= 414.

Density: 1.66 at 27°C.

Vapor pressure: 0.17 to 0.40 mmHg at 25°C
3.0 to 4.0 mmHg at 90°C

Heat of fusion: 0.39 cal/g

Specific heat: 0.258 cal/g/°C at 41°C

Viscosity: 1.4 poises at 100°C

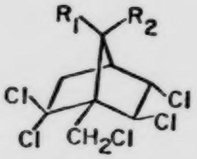
A considerable interest in the chemical constituents of toxaphene has resulted from the desire to understand and identify those components which exhibit toxic properties. Within the last 3 years at least thirteen reports of attempts to fractionate toxaphene have appeared (5, 6, 7, 8, 9, 13, 14, 15, 16, 17, 18, 19, 20) and indications are that this work will continue.

More than 177 C_{10} polychlorinated derivatives are evident on analysis by column fractionation, followed by combined gas chromatography -chemical ionization mass spectrometry. No constituent makes up more than a few percent of the technical mixture and most of the components are probably isomeric hepta-,octa-, and nonachlorobornanes (5, 6, 7). The first constituent chemically identified (by X-ray crystallography and NMR) was 2,2,5-endo,6-exo,8,9,10-heptachlorobornane (6, 20), now known as Toxicant B. Subsequently, a dihydrocamphene component was identified and was reported to be present in technical Toxaphene at a concentration of about 0.8% (8, 9).

Structures have been proposed for the most toxic fractions identified, Toxicant A (16), which appeared by NMR spectroscopy to be primarily a mixture of two compounds present in a ratio of 60:40, which together made up 80% of Toxicant A. The remainder of Toxicant A was a mixture of contaminants in which no compound was present at a concentration of more than 1 - 2.5 ppm. The two main components of Toxicant A, 2,2,5-endo,6-exo,8,8,9,10-octachlorobornane and 2,2,5-endo,6-exo,8,9,9,10-octachlorobornane, were designated A-1 and

A-2 (16) and alternatively, A-α and A-β (17), respectively (Fig. 3). A total of eight components of toxaphene have been isolated. Three of their structures are known, while structures for three others, known as compounds I, II & III, have been proposed (14).

FIGURE 3. Toxic Components Identified in Toxaphene



R,	R,	Designation
CH_2Cl	$CHCl_2$	toxicant A-1
$CHCl_2$	CH_2Cl	toxicant A-2
CH_2Cl	CH_2Cl	toxicant B

(from Reference 13)

2.4 Nature And Content of Commonly Associated Impurities

As was discussed in detail in section 2.2 and 2.3, toxaphene is a mixture of some 177 to 200 components. It is probable that the complete range of toxic components in technical toxaphene has not been characterized. Therefore, the full spectrum of non-toxic impurities present is not known with certainty.

2.5 Analytical Methodology

2.5.1 General Methods

When toxaphene is the only chlorinated material present, classical analytical methods for total chlorine estimation, such as sodium-isopropanol or sodium biphenyl methods, have been applied with the liberated chloride being precipitated with silver nitrate and estimated gravimetrically, or the excess silver nitrate determined by titration. The sodium biphenyl method has been used both for technical toxaphene and toxaphene dust. Total chlorine may also be determined by the Parr Bomb oxidation method (2).

The infrared spectrum of toxaphene shows prominent absorption bands in the 6 to 8 micron region and, by comparison with an authentic specimen, the specified absorbance at 7.2 microns of 0.0177 (maximum) is used to characterize the material. For its determination in formulations such as dusts or emulsifiable concentrates, the carriers or dispersing agents are removed by passing benzene extracted material through columns of acid alumina. The residue, after solvent evaporation, is then subjected to IR-analysis in carbon tetrachloride, and the difference in absorbance at 7.2 and 7.3 microns measured, which is the difference between the maximum and minimum absorbances. Benzene extraction in these procedures dissolves an insignificant amount of any sulphur present in the formulation. A related method uses the same clean-up procedure but employs the difference in absorbance at 7.7 and 5.8 microns; if DDT is also present, it is determined simultaneously by comparing the absorbance difference at 9.1 and 5.8 microns against a calibration curve prepared using various concentrations of standard DDT (2).

Sodium reduction methods for total chlorine may be applied in residue analysis when the history of the samples is known, so that any extraneous chlorine-containing materials can be allowed for. The sodium-liquid ammonia technique for total chlorine has been adapted to the determination of toxaphene residues in crop extracts and cattle dips and the chloride may be determined by automatic titration using coulometrically generated silver ion (21). The Schöniger oxygen flask method is also applicable and enables multiple analyses to be conducted simultaneously. Adsorption chromatography on Florisil can be used to free samples from plant pigments and waxes before further treatment, and extraction of hexane solutions with mixed concentrated and fuming sulphuric acids has been used to free toxaphene from fats and oils (22).

A spectrophotometric procedure for toxaphene residues involves heating a cleaned-up residue with diphenylamine and zinc chloride at 205°C to give a greenish-blue complex, with an absorption maximum at 640 nm (23). This method enables 20 to 700 micrograms to be detected and has been applied to extracts of crops and foods and to various formulations, including those containing DDT or sulphur. Sulphur interferes with the determination but sulphur contamination of extracts can largely be avoided if methanol is used as the extractant. Cyclodiene compounds also interfere, but these do not normally raise serious problems. Small quantities of waxes and pigments interfere severely, and rigorous clean-up is required for their removal.

Gas liquid chromatography, with electron capture detection, which is discussed in detail in later sections, provides the most sensitive quantitation of toxaphene. A typical gas-chromatogram of toxaphene exhibits about 12 peaks, of which three are particularly prominent (24). In principal, toxaphene residues can be analyzed, as in the case of chlordane, by comparing these "signature peaks" with those in a known amount of reference sample, but this will give an exaggerated value for the residue, since other components disappear at a greater rate (2). Alternately, the area under all or part of the toxaphene "fingerprint" can be compared to a reference scan and the concentration of toxaphene estimated (22).

In 1970, Klein and Link (24) reported that DDT interfered with the thin layer chromatographic and gas liquid chromatographic determination of toxaphene. The electron-capture detector response to DDT was several times greater than an equal amount of toxaphene and the DDT scan overlapped with the toxaphene peaks. Consequently, the degree of interference caused by DDT in the gas chromatographic analysis of toxaphene was disproportionate to the amount of DDT present. Interference could be overcome by treating the sample extracts with cold sulphuric-fuming nitric acids (1+1). The acid treatment converted DDT to compounds which did not register on the gas chromatograph, but toxaphene was not affected. The interference by various other pesticides was also prevented. This acid treatment was also useful in the toxaphene colorimetric method by destroying sample organic background. Using this modification toxaphene levels below 1 ppm could be determined by colorimetry. However, chlordane was not removed by the acid treatment and interfered in both the gas chromatographic and colorimetric procedures. Interfering substances contributed by acid treatment were removed by eluting the solutions through a column of activated Florisil with ethyl ether-petroleum ether (6+94). Average recoveries were estimated to be in the range of 87.5 to 91.7% and the limits of detection appeared to be 1 ppm in mixtures containing equal quantities of toxaphene and DDT (24).

Johnson (25) reported a simple colorimetric method for the detection of toxaphene in agricultural formulations based on its reaction with pyridine and methanolic potassium hydroxide. The method was not particularly sensitive, with the optimum concentration required being between 2 and 6 mg/ml of hexane. Most chlorinated insecticides did not interfere with the test, but the method would appear to have limited applicability.

Toxaphene may be qualitatively identified using a chemical test based on the colormetric reaction with diphenylamine in the presence of zinc dust, which produces a grey-green complex with toxaphene (26).

Faucheux (27), in 1965, reported on the use of the diphenylamine zinc chloride chromogenic agent for detection of toxaphene, DDT, and chlordane on aluminum oxide thin-layer plates. Characteristic colours were obtained for these pesticides and the lower level of detection was about 5 ug. The greyish-green colour produced with toxaphene could be differentiated from any of 34 insecticides and 33 other pesticides tested, except strobane.

A thin layer chromotographic method for the separation of PCB, toxaphene and chlordane components was reported by Ismail and Bonner (28). The sensitivity for toxaphene was in the order of 2.5 ug.

Because of the relatively low volatility of toxaphene, no particular precautions need to be taken to prevent evaporation from samples or extracts. However, liquid samples and extracts should be stored in scrupulously clean glassware with glass or Teflon seals. If plastic or rubber comes in contact with liquid samples or extracts, contaminants are usually extracted which interfere with gas liquid chromatographic analyses, particularly if electron-capture detection is used.

To prevent spoilage, samples should be refrigerated or frozen until analyzed.

2.5.2 Specific Methods

2.5.2.1 Water

In 1969 Schafer et al (29) monitored river water in the United States for toxaphene content. Using gas chromatographs equipped with early model electron-capture detectors, a precision of ± 0.01 ng per 5 ul was attainable for solutions of the commonly used organochlorine pesticides in concentration ranges from 0.1 to 1 ng per 5 ul (20-200 ng/ml). These investigators were able to rely on samples of water which were either 100 ml or 1 gallon in size. Pesticides were extracted from the water into hexane without a transfer step and were concentrated if necessary. At 2.5 ppb the authors reported that recoveries were 94.7 ± 11.5%.

Water samples were extracted by Terriere et al (30) using redistilled hexane (Skellysolve B) in a ratio of water:Skellysolve of 2:5. The hexane extracts were dried with anhydrous sodium sulphate, concentrated to 5 ml on a steam bath and transferred to blood sedimentation tubes. The samples were further concentrated at room temperature under air to a volume of 0.1 ml. Suitable aliquots of the concentrated solutions were introduced into a gas chromatograph for analysis. These authors used a gas liquid chromatograph with an electron-capture detector sometimes coupled to a microcoulometric system, which gave improved sensitivity.

They were able to detect 0.2 ug/l (ppb) of toxaphene in various types of samples, with recoveries of about 80%.

Nicholson et al (31) sampled water using a carbon adsorption method and used a procedure which permitted the recovery of dissolved organic compounds from a volume of water ($\sim$ 19 m^3) which was large enough to yield sufficient extracts for chemical analysis and bioassay. The carbon was taken out of the cylinder, dried and extracted with chloroform for 24 hours in a Soxhlet apparatus. The chloroform was evaporated, the residue taken up in acetonitrile and the insecticides partitioned into petroleum ether. The ether fraction was washed with distilled water, dried over sodium sulphate, transferred to a Florisil column and eluted with a mixture of 15% diethyl and 85% petroleum ether. The ethers were volatized and the residue dissolved in benzene. Samples were analyzed with a gas chromatograph using a microcoulometric titration detection system, which was reported as having a limit of detection of 9 ppt in water. These investigators also used infrared spectroscopy and paper chromatography and noted that these latter two methods, by themselves, did not give a definite indication that the sample being analyzed contained toxaphene.

Ground water, which was decanted, filtered and stored at about 5°C, was analyzed by LaFleur et al (32). Analysis for pesticide residues was done as soon as practicable (1-10 days), but re-assay 1-10 months later indicated that toxaphene was stable under the storage conditions used. Ground water toxaphene was recovered by multiple extraction at room temperature with petroleum ether and assayed by EC gas chromatography. The methods used detected as little as 0.012 ± 0.004 micromoles per litre (ca 5 ug/l) of toxaphene in ground water.

Bradley et al (33) took 4 litre samples of water in 9-litre glass bottles with ground glass stoppers. Five hundred ml of a solution of n-pentane and diethyl-ether (3:1 v/v) were added to each bottle, and the contents shaken vigorously for 15 minutes on a mechanical shaker followed by siphoning of the extraction solvent into a tubular container. The extraction was repeated three times for 5 minutes each with an additional 500 ml of extraction solvent. The extracts from a single sample were combined and filtered through a layer of anhydrous sodium sulphate into a Kuderna-Danish evaporating dish. The volume was reduced to about 10 ml prior to column chromatography.

Clean-up was identical for water, sediment and soil. All of the extracts were transferred into a 10 cm column of activated Florisil which was topped with a 1 cm layer of anhydrous sodium sulphate. Insecticides, including toxaphene, were eluted with 200 ml of 6% diethyl ether in petroleum ether. The volume of the column eluate was reduced and n-hexane was added to make the final volume 10 ml. These solutions were then used for gas chromatography.

Toxaphene was measured with a gas chromatograph equipped with an electron-capture (tritium foil) detector. The 0.3 x 173 cm glass column was packed with 4% SE-30 and 6% QF-1 on Gas Chrom Q (60/80 mesh). Temperatures of the injection port, column, oven and detector were 220, 165 and 205°C, respectively. Toxaphene was calculated from the heights of the three major peaks having elution times different from those of DDT isomers. The lowest concentration of toxaphene identified was 0.1 mg/l (ppm) in soil and 1 ug/l (ppb) in water.

Hughes et al (26) preserved their water samples in a refrigerator in bottles containing a small amount of mercuric chloride. For analysis, the water was filtered and extracted with redistilled hexane. They found that their gas liquid chromatographic-electron-capture method permitted the detection of 2.1 ug/l (ppb) of toxaphene in water.

Sample clean-up was effected by use of adsorbant columns of sodium sulphate over Celite:magnesium oxide (1:1) and Florisil. The eluates were analyzed by gas liquid chromatography on columns of 2.5% DC-200 on Chromosorb W using operating temperatures of: column, 210°C; injector, 180°C; and detector, 210°C. Recoveries were 75% at 5 ug in water and a significant response could be obtained with 1.5 ng of toxaphene.

Dip samples of water in 1-gallon brown glass bottles were collected by Ginn and Fisher (34) and refrigerated until the time of extraction. All analyses were completed within 30 hours after collection. A 1500 ml sample was extracted in a separatory funnel by shaking for 5 minutes with 100-150 ml petroleum ether. The water and petroleum ether were allowed to separate and the petroleum ether collected in an Erlenmyer flask. Extraction was repeated three times. The aqueous phase was discarded and the petroleum ether phase dehydrated with anhydrous sodium sulphate. The extract was reduced to a known volume over steam and no further clean-up of the extract was necessary prior to gas liquid chromatography with electron-capture analysis. Glass columns (6 foot) were used of various silicon liquid phases, including 3% DC-200, 5% QF-1 or a 2:1 mixture of 5% QF-1 and 5% DC-200, or a 3:1 mixture of 3% DC-200 and 10% QV-17. Nitrogen was the carrier gas and the column temperature was 190°C with the injector being 250°C and the detector temperature 215°C. Analysis was confirmed using two liquid phases of differing polarity, such as DC-200 and QF-1.

In general, the methods reviewed were similar in most respects to those used for analysis of other organochlorine pesticides. Details of clean-up, column liquid phases used as well as gas liquid chromatographic operation parameters, varied from laboratory to laboratory. Each method is probably satisfactory for the purpose reported but it appears that those of Hughes et al (26) and Nicholson et al (31) should provide sensitive, reliable analysis of toxaphene in water.

2.5.2.2 Food

Residues of toxaphene in foodstuffs can be determined by gas liquid chromatography after removal of interferences by passage through a sulphuric acid-Celite column and dehydrochlorination (11, 35).

The sulphuric acid removes fats and oils, and the dehydrohalogenation procedure gives a characteristic, reproducible pattern for dehydrohalogenated toxaphene (35). In this method, the sample is eluted from the Celite column with redistilled hexane followed by dehydrohalogenation using potassium hydroxide at a temperature of 75 - 80°C. Suitable gas liquid chromatographic conditions use an electron-capture detector and a column of a 1:1 mixture of 5% SE30 and 5% DC-710 silicon on Gas Chrom Q. A column temperature of 200 - 210°C, detector temperature of 190 - 200°C, and injector temperature of 225 - 230°C provide satisfactory performance. It is necessary to inject a standard of dehydrohalogenated toxaphene prior to each injection of sample into the gas liquid chromatograph, which considerably reduces the number of samples which can be processed each day. Column conditioning is very important, and the column should be conditioned for at least 2 days at 250°C while passing carrier gas through.

If additional clean-up is necessary, a standard Florisil column using 6% ethyl ether in hexane for elution may be employed. Strong acid treatment and Florisil column clean-up do not change the gas liquid chromatographic and infrared patterns of toxaphene.

For the analysis of unmodified toxaphene, a column of 3.8% UCW-98 on Diatoport S with a column temperature of 150°C, detector temperature of 205°C and injector temperature of 235°C has been used (35).

If DDT interference is a problem, acid treatment and Florisil column clean up of sample extracts, as described in section 2.5.1, can be used, but results in some loss of sensitivity.

Raw potato tissue was homogenized in water and extracted with benzene (36). Potato extracts carried a natural toxicant which interfered with the assay. Studies on the elimination of this interference revealed that pretreatment of the benzene extracts with 0.2% sodium hydroxide removed it without any apparent change to the insecticide residue present. Limited tests suggested that the appending constituent was an unsaturated fatty acid, probably linoleic acid. Terriere and Ingalsbe (36) used these extracts in a mosquito toxaphene bioassay. The lowest level of detection of toxaphene was reported as being 0.04 mg/kg (ppm).

Hawthorne et al., (37) in 1974 sampled catfish (edible portion only) which was ground in a Waring blender with 500 ml of a 3:1 mixture of hexane:isopropyl alcohol, blended for 2 - 3 minutes and transferred to a rotator and mixed for 2 hours. A subsample was filtered through prewashed glass wool and the isopropanol washed out. The final solutions were dried by passing through anhydrous sodium sulphate and the extracts concentrated to yield two 15 ml aliquots. One of the 15 ml aliquots was partitioned three times into an acetonitrile hexane mixture. The acetonitrile solution was dried and extracted with hexane. The hexane solution was passed through a Florisil column, concentrated and analyzed by a gas liquid chromatograph with electron-capture detector on 1.5% OV-17 plus 1.9% QF-1 on Gas Chrom Q or 3% DC-200. The sensitivity of the method was of the order of 0.01 mg/kg (ppm) and recoveries were 90 - 100%.

It was recommended (38) for food analysis that toxaphene residues be dehydrohalogenated with potassium hydroxide and measured by gas chromatography using an electron-capture detector. As noted earlier, the clean-up procedures and dehydrohalogenation removes interfering residues, except chlordane.

Thin-layer or paper chromatographic methods are useful, but they are somewhat difficult to use in surveillance work. Consequently, gas chromatographic methods are recommended.

2.5.2.3 Air

Stanley et al., (39) examined air for toxaphene content using a special sampler. The collection train (sampler) consisted of a filter and impinger with a trapping liquid, and an adsorbent. The filter was unimpregnated glass cloth about 0.5 mm thick. A Greenburg-Smith impinger, modified by the addition of extra O-rings at the inlet and outlet, was used. The trapping liquid was hexylene glycol which was purified before use by passage through a charcoal column. The adsorbent was alumina, which had been heated to 545°C for 2 hours before use, and was held in the tube by a glass wool pad at each end. The alumina was re-used after ignition, but the filter and hexylene glycol were discarded after use. Sampling was for either 12 or 24 hours for a total of 7 days each at a flow rate of about 29 litres/minute, giving an air sample of about 40 m^3 in 24 hours. Gas liquid chromatography with electron-capture detection was carried out using glass columns packed with 5% SE-30 on 100/120 mesh Gas Chrom Q, or 2.5% SE-30 - 3.5% OV-17 on 100/120 mesh Gas Chrom Q.

In a study of the atmospheric content of toxaphene over the North Atlantic by Bidleman and Olney (40), chlorinated hydrocarbons were sampled by drawing air through plugs of polyurethane foam. The pesticide remained mainly in the foam plug, from which it was eluted prior to separation from PCB, DDT and chlordane on silicic acid columns. Dichloromethane was used to elute the toxaphene from the silicic acid followed by gas chromatographic (with electron-capture detector) analysis on 1.5% OV-17 - 1.95% QF-1 and 4% SE-30 - 6% QF-1 columns. Confirmation of toxaphene was carried out by treating the samples with acid (35). This method was responsive to as little as 3.0 nanograms in a 1,000 m^3 sample. The lowest level of toxaphene detected was 62 ng/m^3.

While DDT and DDE were among a number of pesticides that were analyzed by these authors, there was no comment indicating that these chlorinated compounds caused problems in the identification and quantification of toxaphene.

2.5.2.4 Aquatic Biota

Duke and Wilson (41), in 1971, examined toxaphene residues in fish tissue. A composite sample of liver was mixed with a dessicant of 9 parts anhydrous sodium sulphate and 1 part microfine precipitated silica and mixed until a free-flowing powder was obtained. Samples were extracted with petroleum ether in a Soxhlet apparatus, the extracts concentrated and partitioned into acetonitrile. The acetonitrile was evaporated to dryness at room temperature and the residue passed through a Florisil column. The lower limit of sensitivity was given as 0.01 mg/kg (ppm) on a wet-weight basis.

Fish and mollusks (oysters, clams and mussels) were analyzed by Butler (42) for toxaphene who used similar methods to Duke and Wilson (41). Three columns of different polarity, DC-200, QF-1 and mixed DC-200/QF-1 were used for identification and analyses. However, it was noted that toxaphene sometimes interfered with the quantitation of DDT. The lower limit for toxaphene was 250 ug/kg (ppb).

In 1973, Hughes and Lee (43) extracted toxaphene from fish with organic solvents followed by clean-up using various column chromatographic methods and gas chromatographic-electron-capture analysis. The mass of toxaphene in the sample was determined by comparing its peak area, using a planimeter, to curves prepared from standard solutions of toxaphene. The presence of toxaphene in the sample extracts was confirmed by thin layer chromatography.

A variety of specimens were analyzed for toxaphene content by Terriere et al (30). Aquatic plants, without drying, were passed through a food chopper then tumbled end over end for an hour with hexane:isopropyl alcohol (1:1). The solvent was removed by decantation, washed free of alcohol, and dried with anhydrous sodium sulphate. Samples of aquatic invertebrates or fish were macerated in a Waring blender in the presence of anhydrous sodium sulphate. This mixture was then further macerated for 5 minutes with a 9:1 mixture of hexane and ethyl ether and the extract dried with sodium sulphate. Extracts of aquatic plants, equivalent to 25 grams of plant material, were passed through a column filled with 10 grams of a 1:1 mixture of magnesium oxide (Sea Sorb 43) and Celite 454. The columns were eluted with 125 ml of a 94:6 petroleum ether: ethyl ether mixture (30). Extracts of fish and invertebrates (equivalent to 5 grams of tissue) and bottom mud (equivalent to 25 grams) were purified by passage through a sulphuric acid-Celite column and eluted with hexane. Suitable aliquots of concentrated extracts were analyzed by gas liquid chromatography. Improved sensitivity was obtained by using a short micro-coulometric column with detection possible at toxaphene concentrations of 0.2 ug/kg (ppb) in the various types of samples analyzed, with recoveries of about 80%.

Hannon (44) extracted toxaphene from water, bottom sediment, plankton and fish. Methods of extraction of the insecticide residues from water were taken from Briedenbach et al., (45). Bottom sediments were extracted with hexane:propanol-2 (3:1) and plankton with acetonitrile. Lipids were removed with 1:9 v/v ethyl ether and petroleum ether. Insecticides, including toxaphene, were purified from the lipid extracts for gas liquid chromatography - elctron-capture analysis using a Florisil column procedure. The authors reported minimum analytical confidence limits, in ug/l or ug/kg (ppb) of fish, 20; plankton-algae, 5; water, 1; bottom sediment, 10. The average recovery (± 10%) of toxaphene ranged from 61% in bottom sediment to 85% in water. Two columns were used, including 5% DOW-11 silicone and 2% AF-1 silicone, both on 60/80 mesh HMDS treated Chromsorb W.

While studying the fate of toxaphene in several Wisconsin lakes, where the pesticide was used for rough fish control, Hughes et al (26) developed special clean-up procedures for water suspended solids and various lake organisms. They commented that the amount of clean-up of samples necessary from different lake systems differed. All samples were collected and frozen.

Water and plankton were extracted directly with hexane, whereas fish were partially digested on a steam bath in a mixture 1:1 perchloric acid:acetic acid prior to hexane extraction. For lake sediments, a mixture of hexane and acetone (41:59 v/v) was used. Generally, extracts were freed of acetone by evaporation and the hexane extracts passed through a column of sodium sulphate:Celite, magnesium oxide and Florisil. Gas liquid chromatographic analysis was carried out using columns of 2.5% DC-200 on Chrom W. The electron-capture detector showed significant response to 1.5 ng of toxaphene. Recoveries from all types of samples were 75 - 90%.

Detectable levels were 1.2 ug of toxaphene per gram (ppm) of fish, 250 ug/gram of plankton, 217 ug/gram in suspended solids, 2.1 ug/l (ppb) in water and around 1 ug/g (ppm) in sediment. The detectable limit for toxaphene in fish was as low as 0.2 ug/g (ppm), depending on the presence or absence of other organochlorine pesticides. Toxaphene could be detected at 0.5 ug/g (ppm) in lake sediments. This study demonstrated that the methods generally used for clean-up of environmental samples by gas liquid chromatography - electron-capture analysis of pesticide residues do not always result in adequate clean-up of samples from different lakes. Each lake system should be treated individually and clean-up procedures modified to remove the interference present in the aquatic samples taken from a specific body of water.

Ginn and Fisher (34) also analyzed phytoplankton, fish, soil and water. Samples were frozen at 4°C or quick-frozen at -20°C until extracted and analyzed, blended with anhydrous sodium sulphate, frozen and re-ground until a free-flowing powder mixture was obtained. Residues were extracted in a Soxhlet extractor with petroleum ether. Following extraction the petroleum ether was reduced to about 15 ml with steam and partitioned over acetonitrile 2 or 3 times. The acetonitrile was evaporated just to dryness at 40°C. Prolonged drying or excess heating was avoided to prevent loss of pesticide due to volatilization. The residue was resuspended in petroleum ether and subjected to Florisil column chromatography with elution being effected with a 6% solution of anhydrous ethyl ether in petroleum ether, followed by elution with 15% ethyl ether in petroleum ether. The toxaphene, which was in the 6% elute, was reduced to a suitable volume over steam and subjected to gas liquid chromatography.

It would seem that, for sample preparation, storage, extraction and detection, the method of Butler (42) offers utility in the routine analysis of fish and mollusks.

2.5.2.5 Soil and Sediment

Surface water, soil and sediment were analyzed for toxaphene content by Bradley et al. (33). Samples were extracted in a Soxhlet apparatus with isopropanol and benzene (2:1 v/v) with clean-up being identical for water, sediment and soil. Extracts were transferred to a column of activated Florisil, which was topped with a layer of sodium sulphate. The insecticides, including toxaphene, were eluted with 200 ml of 6% diethyl ether in petroleum ether. The volume of the column eluate was reduced and n-hexane was added to make the final volume to 10 ml. Toxaphene was measured using gas liquid chromatography - electron-capture with glass columns packed with 4% SE-30 and 6% QF-1 on Gas Chrom Q (60/80 mesh). Toxaphene was determined from the heights of the three major peaks with elution times different from those of DDT isomers. In samples containing both DDT and toxaphene the latter was determined in the same way as in samples without DDT, and DDT derivatives were estimated by triangulation from the peaks superimposed on the toxaphene curve. The lowest concentration of toxaphene identified was 0.1 mg/kg (ppm) in soil and 1 ug/kg (ppb) in water.

Parr and Smith (46) added toxaphene to dry soil and soil which had been moistened to mimic sediment samples. Soil was extracted with hexane:acetone (49:59 v/v) in a Soxhlet apparatus for 3 hours followed by partitioning of the extracts with distilled water to remove the acetone. The hexane layer was dried with sodium sulphate. Mud and sediment were similarly treated with minor modifications and recovery of toxaphene from dry soil and samples a analogous to sediment, approached 100%. Extracts were assayed by gas liquid chromatography with a ^{63}Ni-electron-capture detector operated at 265°C with an inlet temperature of 220°C and an oven temperature of 205°C. The column was packed with 3% OV-1 on Chromosorb W.

Pesticide residues were recovered from core samples of soil by Ginn and Fisher (34) by triple extraction with acetonitrile. The

1 ug/kg (ppb) of DDT or toxaphene with recoveries of about 90%.

The method of Swoboda et al.,(48), if modified sufficiently (24) to remove interference from DDT, should prove to be highly useful in the analysis of toxaphene in sediment and soil.

2.6 Uses

Toxaphene is widely used as a preharvest insecticide treatment on cotton, cereal grains, oil seeds, vegetables, fruits and nut crops. The major worldwide use is on cotton. Most treatments are by foliar application of sprays, dusts and granules, except for the use of bran baits to control cutworms. Toxaphene is often applied to crops as a mixture with other pesticides. There are no post-harvest uses. Toxaphene also has a major worldwide use for the control of ectoparasites of sheep, cattle (not lactating dairy cattle) and pigs, particularly ticks and mites causing scabies. Treatment is usually by emulsion sprays or dips, or solutions applied topically (11).

Toxaphene is the major chlorinated insecticide in use in the United States today. Current use involves mainly a mixture of toxaphene and methyl-parathion for controlling cotton pests (5, 13). About one third of the toxaphene produced is used for insect control on vegetables, small grains, soybeans, and control of external insects on livestock. It has also been employed extensively in fish eradication programs (18).

Toxaphene is also used for the control of grasshopper infestations. It has been used as a supplement to, and a substitute for, DDT for the control of insects resistant to DDT (49).

Toxaphene sprays are not considered to be safe for household application, nor have acceptable medicinal uses for toxaphene been discovered. At one time, it was believed to be comparable to DDT as a louse powder because of its persistence and resistance to laundering; however, uncertainty as to its acetonitrile was evaporated to dryness and the residue suspended in petroleum ether and subjected to a 2-fraction purification on Florisil with elution of toxaphene with 6% ethyl ether in petroleum ether.

Gas chromatographic analysis used 3% DC-200, 5% QF-1, a 2:1 mixture of 5% QF-1 and 5% DC-200, and a 3:1 mixture of 3% DC-200 and 10% OV-17. Analysis was confirmed using two liquid phases of differing polarity, such as DC-200 and QF-1. If was questionable whether the clean-up procedures were satisfactory, as toxaphene quantitation was not possible due to interferences.

Carey et al. (47) pooled soil sample cores and sieved them to a size of 2 mm. Moistened soil was extracted with 3:1 heptane: isopropanol, the alcohol removed by water washing and the heptane extract dried through sodium sulphate. Analyses were performed on a gas liquid chromatograph equipped with a tritium electron-capture detector using a multiple-column system employing polar and non-polar phases such as 9% QF-1, 3% DC-200 or a mixture of 1.5% OV-17 and 1.95% QF-1 to identify and confirm the pesticides. Sensitivity (minimum detectable levels) for organochlorine compounds ranged from 0.002 - 0.03 mg/kg (ppm), except for mixtures of polychlorinated biphenyls (PCB's), chlordane, toxaphene, etc, whose minimum detectable levels were 0.05 - 0.1 mg/kg (ppm). The average recovery from soil was 90 - 110%.

Swoboda et al.,(48) took soil samples which were air dried, sieved, mixed, placed in half gallon plastic containers and stored for up to 6 months at room temperature. Soil was extracted with 1:2 benzene:isopropanol by shaking for 2 hours and then filtered through a fritted glass filter. The extract was passed through a Florisil column and eluted with 6% ethyl ether in petroleum ether. Samples were evaporated to near dryness at room temperature and injected into a gas liquid chromatograph equipped with an electron-capture detector. A 6-foot column of 10% DC-200 on Anakrom ABS was used with a column temperature of 205°C. The authors reported that the limit of detection was greater than cutaneous toxicity in powder precludes its use for this purpose. In medical entomology, however, it is proving to be a valuable agent for the control of ticks and mites which transmit disease (50).

Toxaphene has proved valuable for a number of purposes for which DDT was not particularly well suited, although toxaphene's general toxicity is greater than that of DDT. Toxaphene does not cause tainted crops and has the ability to reach insects in inaccessible situations where DDT is ineffective. Toxaphene is often effective when target insects are heavily sclerotized. Toxaphene's action seems to be a combination of stomach and contact poisoning and it is, therefore, an effective control agent for grasshoppers in the field. Various pentatomid and mirid plant bugs are better controlled by toxaphene than by DDT and several species of thrips, especially when found on cotton, are also well controlled by toxaphene (2).

It is evident that in field practice, toxaphene ranks with DDT and other organochlorines as a toxicant for many of the lepidopterous larvae infesting agricultural crops. Toxaphene has proved to be useful against tortricid caterpillars on fruit (as a 0.1% spray), but is less effective against red banded leaf rollers and will not control the codling moth. Toxaphene granules and dusts have given adequate control of various boring larvae, such as the sugarcane borer, and has the advantage over DDT of not causing aphid infestations. Toxaphene, as a 0.2% spray, gives good control of psychid moth larvae (Bagworms). As a soil insecticide, toxaphene appears to be generally inferior to the cyclodienes, probably because it tends to disappear from soil more rapidly than other organochlorine insecticides; nevertheless, a report in 1949 indicated that 18 months after an application of 25 pounds per acre, it was effective against larvae of the Japanese beetle.

Toxaphene has the ability to control the main cotton pests moderately well and has proved valuable for this purpose, especially in combination with DDT, which by itself is relatively ineffective against the boll weevil. A 2:1 toxaphene:DDT combination has proven very useful (at 1-6 lbs/acre) in the control of the boll weevil, leaf-eating caterpillars, various leaf hoppers, plant bugs and thrips. Along with other polychloroterpenes, toxaphene possesses some degree of acaricidal activity, but it is frequently formulated with sulphur for cotton applications due to its tendency to provoke mite infestations, presumably through predator reduction. It is also efficacious against various species of orchard mites.

As a mixture of compounds containing chlorinated bridged rings, toxaphene is frequently classified with the cyclodienes, although its origins are different. Nevertheless, there is clearly a general resemblance between such molecules and the hexachlorobornane nucleus of the cyclodienes, which appears to be reflected in the fact that the cyclodiene-resistance extends to toxaphene. Toxaphene resistance in the boll weevil and some other cotton pests became widespread from about 1955 onward, which explains why the synergistic combination with DDT has proven to be so valuable (2).

3. SOURCES OF ENVIRONMENTAL POLLUTION

3.1 Natural Occurrence

Toxaphene is a synthetic chlorinated hydrocarbon insecticide which does not occur naturally in environmental media. Its presence in the environment can be correlated with prior agricultural use through foliage sprays, dusts or insect baits. Its presence in soil, air and water is related to either manufacturing processes or crop applications. (52).

3.2 Synthetic Production

No data were obtained which documented the quantities of toxaphene manufactured or imported into Canada. Toxaphene is registered for use in Canada under controlled conditions (pers. commun.,1977)* Hercules Canada indicated that toxaphene is not sold in Canada except on special request. Toxaphene is not manufactured in Canada and any which is used is imported from the U.S.A.

The original patent for the manufacture of toxaphene (4) was used by Hercules Inc., Brunswick, Georgia. Toxaphene (camphechlor) is currently manufactured at four locations in the U.S.; Sanford Chemical Co., Houston, Texas; Tenneco Inc., Fords, New Jersey; Vicksburg Chemical Co., Vicksburg, Mississippi and Hercules Inc., Brunswick, Georgia.

Although Hercules Incorporated declined to furnish its production figures for toxaphene to the U.S. Environmental Protection Agency, it offered no evidence to contradict estimates of 50 - 80 million pounds per year used by the Environmental Protection Agency in computing its proposed mass-emission standards (53).

Other estimates of U.S. production figures were 70 - 90 million pounds in 1972 (54) and 50 million pounds in 1975 (17).

3.3 Utilization Patterns

3.3.1 Extent Of Use

3.3.1.1 By Industry

It is most probable that essentially the entire production of toxaphene is used by the agricultural industry, mainly to treat cotton crops in the southern U.S.A. Toxaphene is generally applied at the rate of 1.26 - 3.78 kg/hectare for insect control.

(*Can. Dept. Industry Trade & Commerce).

It is frequently used in the U.S.A. in combination with methyl parathion, parathion or other insecticides (54).

Since its first use in 1946, world-wide consumption has been estimated to be over one billion pounds (55).

The total consumption of toxaphene in the U.S.A. was estimated by Rensberger (56) to be about 37 million pounds in 1971. During 1974 the estimated toxaphene use in the United States was 40 - 58 million pounds (46, 54).

In 1962 only 139,000 pounds of toxaphene were used in Alabama (31). However, in 1971 about 4.5 million pounds of toxaphene were applied to suppress insects on cotton and soybean cropland (57) and in 1975 toxaphene was the most widely used chlorinated insecticide in the U.S.A. (17).

As pointed out in section 3.2, toxaphene is not in general use in Canada for agricultural or other purposes.

3.3.1.2 By Research Establishments

No quantitative estimates of the use of toxaphene in research were available but the quantities used for chemical and toxicological studies are probably insignificant, when compared to the total production.

3.3.1.3 By The General Public

At one time toxaphene was available in the U.S.A. in domestic sprays. However, this practice was discontinued around 1952 and, therefore, it is probable that consumption by the public is virtually nil (50). Toxaphene has never been available in Canada in forms suitable for consumption by the public.

3.3.2 Industrial Release To Air and Water

3.3.2.1 Intentional

In Canada there would be no intentional release of toxaphene to the environment, except when it is used in special instances, which according to Hercules Canada is rare.

In the U.S.A. toxaphene is applied to crops mainly by aerial spraying and so the potential for wide spread distribution exists. In addition, toxaphene has been used to kill "rough" fish in lakes and water systems and in these instances the pesticide is added directly to the water. Basically, the entire U.S. production of over 50 million pounds annually is released into the environment by way of these two routes.

3.3.2.2 Accidental

No reports of accidental release of toxaphene to the environment were found in the literature.

Obviously, unintentional drift onto non-target areas during aerial application is always a hazard and may affect land as well as water biota.

3.3.2.3 Waste Disposal

Because toxaphene is not manufactured or used extensively in Canada the disposal of waste pesticide is not a concern.

In the U.S.A. few reports were reviewed on the disposal of toxaphene, except for those of Reimold (58, 59, 60).

Durant and Reimold (58) found that the sediment of a stream into which a toxaphene plant outfall emptied contained toxaphene up to a concentration of about 2000 mg/kg (ppm). Dredging of the stream to widen it did not result in any increase in toxaphene in oysters, which was as high as 6 ppm, collected 2 miles from the outfall.

In a further study Reimold and Durant (59) found that the toxaphene concentration in the fauna and flora of the creek was higher during dredging than before or after. The best indicator for toxaphene contamination was found to be the marsh grass, which appeared to translocate the toxaphene from the sediments in the creek into the plant tissues. The authors noted that at no time were fish observed to be dying or in signs of distress. High levels of toxaphene were found in anchovy, shrimp and mummichog.

Reimold and Shealy (60) investigated the pesticide and heavy metal content of fish collected from 11 estuaries representing all the Atlantic drainage basins in Georgia and South Carolina. The study was conducted between the fall of 1972 and the spring of 1974. The absence of toxaphene in any of the yearling fishes sampled was extremely interesting to the authors. A toxaphene manufacturing plant at Brunswick, Georgia was in operation throughout the monitoring programme and agricultural use of toxaphene continued in the watersheds of South Carolina and Georgia. Earlier studies had shown detectable quantities of toxaphene in the estuarine biota during 1971 - 1972. Discrepancies between the toxaphene residues measured in the two studies suggested that a pollution abatement programme, initiated by the toxaphene manufacturing plant, had effectively removed toxaphene from the biota prior to the later survey.

3.4 Other Means Of Production

Toxaphene is not formed in the environment as the result of chemical degradation of other chlorinated hydrocarbons.

3.5 Availability & Effectiveness Of Control Technology

The normal alum coagulation treatment of drinking water does not remove significant amounts of toxaphene (31). Carbon adsorption is effective in removing toxaphene from water, but is expensive and the binding capacity is limiting (61).

Cohen et al., (61) found (by serial dilution of toxaphene) in Cincinnatti tap water that the threshold odour concentration was 0.0052 mg/l. It was also found that treatment of a solution of 2 mg/l of toxaphene with 10 mg/l of activated carbon removed toxaphene, but left a residue of 0.76 mg of toxaphene per litre. The authors provided an adsorption isotherm for toxaphene and calculated that at 0.2 mg/l of toxaphene, 10 mg/l of activated carbon would be required to obtain a threshold odour number of 1. Any concentration of toxaphene over 0.0052 mg/l provided an odour to water which was unacceptable. It was concluded that toxaphene could be removed by treatment with activated charcoal, although fairly large quantities were required.

Based on an annual production of 50 million pounds of toxaphene a year, Hercules Incorporated, of Brunswick, Georgia estimated the cost per pound to install a reductive degradation toxaphene waste water treatment system to be $0.0031 per pound. If an activated carbon adsorption system were used, it would increase the cost to $0.0047 per pound of toxaphene product. The most expensive treatment, resin adsorption plus reductive degradation, was estimated to cost in the area of $0.0082 per pound (53).

It was estimated that reductive degradation by itself would reduce toxaphene in the treated effluent from toxaphene-producing plants to less than 0.0072 pounds per day, whereas activated carbon adsorption would reduce the toxaphene outflow to less than 0.018 pounds per day. Resin adsorption plus reductive degradation would reduce the toxaphene content of plant effluents to 0.0002 pounds per day, giving a plant effluent toxaphene level of less than 0.1 ug/l (ppb). Carbon adsorption could only reduce the toxaphene content in the effluent to less than 5 ug/l and reductive degradation to less than 3 ug/l (53).

It was estimated that a waste water treatment system based upon reductive degradation technology, with a design capacity of up to 300 gallons per minute waste water flow, could be designed and installed so as to commence operation within a period of one year. This one-year period included bench-scale testing in the laboratory, testing at the toxaphene manufacturing site, design, installation and full operation, including the working out of any start-up problems. This system could achieve 1.5 ug/l of toxaphene in an effluent within a one-year period. It was stated that since considerable information was available regarding toxaphene degradation, that only 90 days would be needed to put together and assemble the system for toxaphene waste water treatment. Other estimates for the complete installation and operation of a toxaphene waste water treatment system ran as high as 3.5 years for the installation of a plant which would achieve monthly average toxaphene concentrations in the plant effluent of 1.5 ug/l (53).

4. Environmental Distribution And Transformation

4.1 Routes And Mechanisms Of Transport Into The Various Environmental Media

4.1.1 Water

Toxaphene can make its way into water systems via four routes. One is the direct application for piscicide purposes, to reduce to level of "rough" fish.

A second is drift into water when applied to crops from airplanes. Quantitation of the extent of contamination by this route was not available.

The third route includes the toxaphene contained in water run-off from treated land and the fourth is the toxaphene in industrial waste discharges at manufacturing and packaging sites.

Young and Nicholson (62) indicated that in 1950 in northern Alabama, where 15 instances of heavy fish kills occurred in streams and tributaries of the Tennessee river, that the most likely cause was the washing of insecticides from the soil into streams by heavy rains. Heavy applications of insecticides had been made in the area to control the boll weevil prior to the summer of 1950.

The opinion was expressed by Nicholson (52) that the two principal sources of water contamination by pesticides were run-off from the land and discharges of industrial waste. However, other causes of contamination could include activities intended to control aquatic life (plants, fish or insects), carelessness and accidents (55, 63, 64). Studies were carried out in a cotton-growing area in northern Alabama where water was sampled at a municipal water treatment plant situated at the downstream end of the river basin. They found that insecticides ran off the land and entered the river from the watershed in general, rather than from a favourably located cotton field. Toxaphene (as well as DDT and BHC) were recovered in water samples in concentrations generally less than 1 ug/l with highest recoveries being made during the summer, which is the season of application. Nearly all water samples studied contained insecticides year-round, but were greatest during the application period. After insecticide treatment had been stopped the frequency of negative water samples increased with time indicating an improvement in riverwater quality with diminished insecticide usage. DDT exhibited a marked affinity for sediment and suspended sediment was the primary vehicle for its transport. Toxaphene, however, was found much less frequently in association with sediment and appeared to be transported primarily in solution in the water. The authors commented that the potential for pesticide contamination of ground water was much less than for surface water, but it could occur. No data were presented to show that toxaphene entered the ground water in their study area.

Nicholson et al.,(31) also reported on insecticide contamination levels in a stream in Alabama from the summer of 1959 through the winter of 1962-63. The stream drained a 400 square mile watershed in which approximately 15,000 acres were cultivated annually. Insecticide usage was estimated to be of the order of 139,000 pounds in 1962 with toxaphene, DDT and BHC comprising over 90% of the insecticides used. Insecticides were recovered from water by use of the carbon adsorption method which permitted recovery of dissolved organic compounds from a large volume of water (about $19m^3$). Between 1959 and 1962 approximately 105 tons of toxaphene were used in the area. Water was sampled from the Flint Creek in Alabama and at the sewage treatment plant receiving this water. Toxaphene concentrations in the range of 7 - 270 ng/l (ppt) were found in untreated and 5 - 410 ng/l (ppt) in treated water. These data showed that the purification processes used at the treatment plant failed to remove toxaphene (similar data was found for BHC). The data suggested that the soil-persistent insecticides in the stream were related to levels of usage and to the solubility of the compound.

Toxaphene was applied to cotton fields in North Carolina during the 1969 growing season at a rate of 26.8 kg/hectre and 0.36% was detected in run-off (65). Of the toxaphene found in the run-off 75% was in the sediment fraction (as compared to 95% for DDT). Toxaphene residues in pond water in the area varied from less than 1 ug/l (ppb) before spring to 65 ug/l (ppb) by about mid-summer. It was concluded that toxaphene was much less persistent in the soil than DDT. Residues of toxaphene in run-off and in soil from the plots were about equal to or less than those of DDT, even though twice as much toxaphene was applied. However, toxaphene posed a serious acute hazard to aquatic fauna in farm plots as evidenced by the high levels found in pond water samples.

Barthel et al.,(66) analyzed the sediment of the lower Mississippi River for various hydrocarbon residues and reported only a few instances of trace amounts of toxaphene being measured. When present, the toxaphene was assumed to have come from the land, since it had been used heavily in the area studied. The main contamination of the Mississippi River was due to the presence of endrin intermediates from manufacturing plants located on the river.

Residues of toxaphene were detected in the waters of a rice field marshland ecosystem, but the investigators were unable to quantitate the levels (34). The toxaphene was present as a result of contamination of the drainage canals during aerial application of an unknown amount of insecticide to a rice field in the study area as a treatment for grasshopper infestation. The toxaphene resulted in a massive fish kill with the number of fish killed being estimated in the tens of thousands and included several species. The population of grass shrimp was so reduced that sampling of the organism was not possible for 6 weeks. Only minute quantities of toxaphene were detected in the water following the fish kill. Furthermore, the limited persistence of toxaphene prevented accumulation of residues in most organisms.

Kallman et al.,(67) studied the fate of toxaphene in a New Mexico lake which had had three treatments at a total calculated concentration of 0.05 mg/l (ppm). Actual surface water concentrations 2 hours after the first treatment were 0.002 mg/l (ppm). After the third treatment, which was 96 hours after the first, surface water levels reached a maximum of approximately 0.028 mg/l. By that time the concentration resulting from the first two applications had been reduced to 0.001 - 0.002 mg/l (ppm). At the end of approximately 31 days concentrations in the surface water were 0.001 mg/l (ppm) or less.

4.1.2 Air

Toxaphene finds its way into the air during aerial application of dusts or sprays on agricultural crops. Toxaphene can remain airborne as an aerosol when it contaminates non-sedimentable particulates in the atmosphere. Toxaphene applied over crops in the southeastern U.S.A. has been found in the atmosphere near Bermuda (40). Toxaphene may also be volatilized from plant and soil surfaces during warm summer temperatures (48, 68).

4.1.3 Soil

Soil contamination by toxaphene arises chiefly from the foliar application of the insecticide to agricultural crops (69).

4.2 Transfer Between Media And Modes Of Degradation

4.2.1 General Movement

When toxaphene is applied to agricultural land the insecticide is usually detectable in the surrounding watershed. Although it is not as persistent as DDT, toxaphene is "bioconcentrated" by aquatic animals and can pose a threat to birds feeding on contaminated fish (56).

The half-residence time of toxaphene in topsoil was noted to be about 100 days (32), although it was found (70, 71, 72) that the half-life could be as long as 6 to 12 years. Contamination of ground water from surface water containing toxaphene was reported.

The movement of toxaphene through soil to underlying groundwater was studied by La Fleur et al.,(32) in plots to which 77uM/kg (32 mg/kg) of toxaphene were applied. It was found that toxaphene loss from the topsoil occurred in two stages. The half residence time in the topsoil was about 100 days and toxaphene was found in the underlying groundwater within 2 months after it was applied to the topsoil. The pesticide persisted in groundwater for at least one year, with concentrations gradually decreasing with time. In one year the topsoil concentration of toxaphene was reduced from 77 uM/kg (32 mg/kg) to 12 uM/kg (5 mg/kg) and underlying soil (approximately 45 -80 centimetres below the surface) contained 2 - 5 uM/kg (0.8 - 2.0 mg/kg) at varying times during the one year study. The groundwater never contained more than 0.14 uM/l (0.058 mg/l) of toxaphene and by the end of one year it had 0.03 uM/l (0.012 mg/l) compared to the one month value of 0.07 uM/l (0.029 mg/l).

Veith and Lee (73) reported on the toxaphene content of sediments from three lakes treated with approximately 0.1 mg/l toxaphene in 1966 - 1967. It was found that toxaphene accumulated to concentrations as great as 90 ug/g (ppm)(dry weight) and the transport to the sediments was attributed to direct adsorption onto the sediment and to co-deposition of toxaphene laden algal blooms with other particulate matter. Toxaphene concentrations in the sediments decreased by a factor of two every 4 months in the three Wisconsin lakes. Toxaphene was transported vertically in the sediments at rates of 0.4 to 1.1 cm/day to the 10 to 15 cm level which was attributed to physical mixing of the sediments. Laboratory studies demonstrated that the adsorption of toxaphene onto sediment was irreversible in aqueous solution and that leaching of appreciable amounts of toxaphene by water was highly improbable.

In a recent survey, Carey et al.,(47) found that toxaphene levels in soils from 14 United States cities were generally higher than levels in the agricultural soils of the respective states. No explanation was offered by these authors in clarification of their findings. The source may have been toxaphene used to control mosquitoes.

Since toxaphene is frequently used with other chlorinated hydrocarbon insecticides, such as DDT (up to 1973), methyl parathion, parathion and naled, with which toxaphene is synergistic, it is to be expected that the synergistic action of these insecticides would be maintained during translocation through the various environmental media. It should be noted that Nicholson (52), reporting on DDT and toxaphene transport in natural waters, stated that "toxaphene was found less frequently than DDT in association with suspended sediment and was transported primarily in solution".

4.2.2 Bioconcentration

Although Ginn and Fisher (34) indicated that the lack of toxaphene persistence in the environment prevented bio-accumulation, there have been reliable reports to the contrary.

Reimold and Durant (59), in studying the contamination of a marsh area with toxaphene, found that marsh grass could be used as an indicator for the presence of toxaphene. In a subsequent study of the same area (74), these authors concluded that the oyster could also be used as in indicator of contamination by toxaphene, since the oyster was a sessile filter-feeder with the ability to concentrate the pesticide up to 70,000 times that of the surrounding water.

Newsom (75) reviewed the consequences of insecticide use as it related to their effects on non-target organisms. He noted that most of the chlorinated hydrocarbon insecticides penetrated plant tissues and were translocated at least to a limited extent. He reported that aquatic invertebrates exposed to 0.63 ug/l (ppb) toxaphene in water had whole-body levels of 1.43 mg/kg (ppm), which resulted in a ratio of storage to exposure of 2270 times. Rainbow trout, similarly exposed to toxaphene at concentrations of 0.41 ug/l, had whole-body concentrations of 7.7 mg/kg which calculated as a concentrating factor of over 18,000 fold. Aquatic plants exposed to 0.41 ug/l (ppb) in water had a resultant toxaphene level in their foliage of 0.21 mg/kg (ppm), or a ratio of storage to exposure of 512.

Experiments to determine the ability of a number of freshwater species to bioaccumulate toxaphene were conducted by F.L. Mayer (53). In these studies, yearling brook trout concentrated toxaphene by a factor of 16,000 and 5,000, at exposure levels of 0.502 ug/l and 0.039 ug/l respectively. After 161 days, the whole-body residues of toxaphene in fish exposed to 0.288 and 0.502 ug/l toxaphene were 2.4 and 8.9 ug/g (ppm), respectively. Such high body concentrations posed a serious latent danger to survival, since extensive mortality was experienced by fish exposed to these levels of toxaphene just prior to and during spawning (53).

Brook trout from all toxaphene exposures (0.039, 0.068, 0.139, 0.228, 0.502 ug/l) were analyzed for the distribution of toxaphene residues between the fillet and remaining tissue (offal) after 161 days. Toxaphene residues in the offal were 2.8 and 4.2 times that found in the fillet. The highest residue detected in the fillets was 4.9 ug/g, whereas residues in the offal were 13.0 ug/g which exceeded the FDA seizure level of 5 ug/g for toxaphene in fish to be consumed by man. The highest concentration factor in the brook trout was found in the fry, which concentrated toxaphene up to 76,000 times the level in the water within 15 days. (53).

Although there is a report to the contrary (56), at water levels known to affect fish (0.04 ug/l) and with an accumulation factor of 20,000, (the accumulation factor given for trout (76)), the resulting residue levels (0.8 ppm) were not expected to approach concentrations known to be a hazard to birds (76).

Goldfish accumulated 200-fold more toxaphene than the surrounding water, in a lake in California. Additionally, a carp species was found to accumulate toxaphene in its fat (1.15 mg/kg) from the surrounding water which contained 0.001 mg/l (ppm) toxaphene (77).

The environmental disposition of ^{14}C-toxaphene in a terrestrial-aquatic model ecosystem was evaluated by Sanborn et al.,(77). The organisms examined included algae, snails, mosquitos and fish and toxaphene was found in every organism at concentrations ranging from 1.4 mg/kg (ppm) in the mosquito to 15.2 mg/kg in the snail. The accumulation of toxaphene by the various organisms of the system calculated as ecological magnification (EM) was 6902 for algae, 9600 for snail, 890 for mosquitoe and 4247 for fish. The authors suggested that any compound with an EM greater than 1000 should be used only under carefully supervised conditions and that toxaphene was definitely in that category with an average EM value of about 4000. It was concluded that toxaphene was a persistent pesticide and had accumulation properties similar to other chlorinated hydrocarbons examined in the same system.

Tests to determine the bioconcentration phenomenon in marine species were conducted by Steven Schimmel at the Gulf Breeze Laboratory, in Florida (53). His tests showed that toxaphene accumulated in whole-body tissues of the longnosed killifish by factors ranging from 4,200 to 60,000 times the water concentration during a 28-day period; concentration in ova ranged from 1,000 to 5,000. In 96-hour studies, bio-concentration factors ranged from 3,100 to 20,600 in the sheepshead minnow; 9,000 to 15,200 in oysters; 400 to 800 in grass shrimp; and 800 to 1,200 in pink shrimp. Pinfish concentrated toxaphene 3800 times the amount present in water (53).

Although the 1976 Estuarine Monitoring Program showed that bioaccumulation in shellfish was occurring in the Georgia area (of 664 samples taken, 128 contained toxaphene, the maximum residue being 54,000 ppb) no toxaphene residues were found in the samples taken from stations off the cost of Georgia. It was recommended that caution should be used in comparing these results to those of earlier years, however, since very few samples had been taken in 1976; and further, those analyzed included only migratory fish species (53).

Toxaphene accumulates in fat of animals after ingestion or after spraying or dipping. There are two important residue characteristics of toxaphene: with any given rate of subacute intake a certain storage level is attained with no build-up above this level; when the source of toxaphene is removed, the residue is rapidly reduced. The level of storage of toxaphene is lower and elimination more rapid than with most other chlorinated hydrocarbons. As a rule, storage levels in the fat of cattle and sheep will be 1/4 to 1/2 the level in the feed. Storage concentrations in hogs is somewhat less than in other livestock, probably because of their greater fat content (69).

4.2.3 Interaction With Other Physical, Chemical And Biological Factors

Clay particles, adsorption to sediment and anaerobic conditions are all favourable for the decomposition of toxaphene (12, 57, 78) whereas urea retards the rate of decomposition of toxaphene (79).

Aquatic studies of toxaphene have been concerned with the influence of temperature, pH, oxygen, water solubility, and adsorption,on its persistence. This organochlorine insecticide is stable in strong acids, but in strong bases it has been shown to undergo dehydrohalogenation. It also loses hydrogen chloride when exposed to ultra-violet light (12).

Hooper and Grezenda (63) found that toxaphene was detoxified most rapidly in laboratory studies when the oxygen content and temperature were high. Toxaphene was detoxified in hard water (alkalinity, 212 ppm) more rapidly than in soft water (alkalinity 6 ppm). These authors (63) also studied the decomposition of toxaphene by microorganisms. They showed that the addition of a physical substrate (glass marbles, gravel, sand, or mud) to the aquarium decreased the toxicity of toxaphene to fish. Sterilization of the substrate with dilute formalin and carbolic acid inhibited detoxification, indicating that microorganisms were responsible for reducing toxicity.

As was discussed in section 3.5, because toxaphene adsorbs to suspended sediment in water, its removal from water will be partially affected by use of sedimentation basins. Also, in water treatment plants where activated charcoal is used, removal of toxaphene will be facilitated.

4.3 Persistence Of Toxaphene And Its Degradation Products

The half-residence time of toxaphene in topsoil was noted to be about 100 days (32) although it has been found (70, 71, 72) that the half-life in soil can be as long as 6 to 12 years.

Terriere and Ingalsbe (36) found that toxaphene could persist in a sandy loam soil for at least 3 years following application at a rate of 10 pounds per acre. Potatoes grown in the soil, however, had residues of less than 0.04 mg/kg (ppm) as determined by both bioassay and chemical methods, which was the limit of detection. The investigators noted that toxaphene appeared to lose a large portion of its toxicity during the 3 year period, as determined using a mosquitoe larvae bioassay.

Alexander (71) indicated a persistence of toxaphene in soil of roughly 6 years. This can be compared to about 10 years for DDT and 9 years for heptachlor. He pointed out that, clearly, these pesticides were either non-biodegradable in soil or were detoxified at such a slow rate as to be considered effectively non-biodegradable. In addition, no microorganism had been isolated which either mineralized or significantly altered the structure of these chemicals.

A report that 45% of an application of toxaphene remained in the soil 14 years after application was published by Nash and Woolson (72). They determined the half-life of toxaphene in soil to be 11 years. The half-life of toxaphene was determined to be long relative to aldrin (5 years), dieldrin (7), chlordane (8), heptachlor (2-4), BHC (2) and DDT (10.5)(2.5-35). The persistence appeared to depend to a certain extent on the concentration of the pesticide in the soil. The authors suggested that conditions were such that the values approached the upper limit of persistence of these insecticides in soil.

Archer (68) applied toxaphene to alfalfa under controlled conditions and then dried the hay by exposure to sunlight, ultraviolet light, or stored it in the dark using only air for drying. He found that maximal loss of residue was 20% when air dried, 46% when dried under ultraviolet light and 54% when dried under sunlight. This loss of toxaphene occurred mainly in the first 7 days of drying, after which time the loss more or less became constant. It was suggested that the loss of toxaphene residues during alfalfa drying was probably due mainly to volatilization, rather than to photodecomposition.

In lake sediments, Veith and Lee (73) found that the toxaphene half-life was about 4 months. Toxaphene was not very persistent on plant surfaces. After 14 days only 1% of the original residue of more than 100 ppm was present on the leaves of kale (2).

Lakes treated with from 0.02 to 0.1 mg/l (ppm) required from 2 - 12 months before they could be restocked. Some lakes required as long as 4 years before restocking was successful (80). Generally, the persistence problem is associated with deep, soft-water, biologically sparse lakes intended for trout fishing (43).

Yap et al studied the application of toxaphene at a rate of 26.8 kg/ha to cotton fields in North Carolina during the 1969 growing season. They concluded that toxaphene was much less persistent in the soil than DDT. Residues of toxaphene in water run-off and in soil from the plots were about equal to, or less, than those of DDT, even though twice as much toxaphene was applied.

Paris and Lewis (12) reviewed the chemical and microbial degradation of various pesticides in aquatic systems including toxaphene. Toxaphene was stable in strong acids but in strong base it was shown to undergo dehydrohalogenation with loss of HCl when exposed to ultraviolet light. These authors claimed that no degradation products had been reported for toxaphene in aquatic systems.

5. ENVIRONMENTAL EXPOSURE LEVELS

5.1 Environmental Levels

5.1.1 Water

Water was sampled at a municipal water treatment plant situated in a cotton growing area of northern Alabama at the downstream end of a river basin. Toxaphene concentrations were generally less than 1 ug/l, with the highest levels being observed during the summer season of application (52).

Schaffer et al.,(29) reported on pesticide levels in raw and finished drinking waters from the Mississippi and Missouri Rivers. The samples were collected between March, 1964, and June, 1967, from ten selected municipal water supplies. No detectable levels of toxaphene were found in any of the samples. The authors used 2.5 ug/l (ppb) in their recovery studies, but gave no indication of their limit of detection for toxaphene in water. It must be assumed that non-detected meant that the levels were less than 2.5 ug/l (ppb). These investigators also presented a table of guidelines for maximum permissible levels of toxaphene in finished waters, which was 5 ug/l in 1965-1966, and 2.5 ug/l in 1967.

In a Report To Congress from the Preliminary Assessment of Suspected Carcinogens in Drinking Water (81), investigations into the contamination of drinking water with pesticides was presented. Although analyses for chlorinated hydrocarbon insecticides and herbicides were carried out from approximately 700 water utilities in the United States, no report was made of the presence of toxaphene in any of the water samples. Other pesticides such as aldrin, dieldrin, and DDT were identified and quantitated. PCB's were measured in water and it may be that toxaphene was masked in the PCB analysis.

It is notable that heptachlorobornane, the most toxic fraction of toxaphene yet isolated, (13) was found in New Orleans drinking water in a 1972 study at a concentration of 0.06 ug/l (ppb)(77).

Between 1959 and 1962 about 105 tons of toxaphene was used on an area of Alabama. Water was sampled from Flint Creek, Alabama, in the area of toxaphene use, and at the sewage treatment plant receiving this water. Toxaphene concentrations in the range 7 to 270 ng/l (ppt) were found in untreated water and 5 to 410 ng/l in treated water. These data showed that the purification processes used at the treatment plant failed to remove toxaphene (31).

During 1970, Hannon et al.,(44) studied the distribution of toxaphene in a large lake ecosystem in a predominantly agricultural region of South Dakota. Although their method was capable of detecting 1 ug/l of toxaphene in water, none was detected.

Bradley et al.,(33) studied a small agricultural area in North Carolina where cotton had been sprayed with toxaphene (26.8 kg/ha). Prior to spraying, pond water in the area contained less than 1 ug/l (ppb), but about two months later 65 ug/l was found in the water. Approximately 60% of the pond watershed was planted to cotton.

As was mentioned earlier in this document (section 3.3), toxaphene is not used to any significant extent in Canada. It is, therefore, unlikely that detectable levels of the pesticide will be found in Canadian drinking water supplies.

In the recently published survey of Organics in Ontario Drinking Water (82) the presence of toxaphene was not reported in any of the samples analyzed. The analytical methods used were such that levels of toxaphene of less than 10 ug/l should have been detected, if present.

The Saskatchewan Department of the Environment informed the author of the present document that they would be publishing residue data by mid-1977. However, no data were available at the time of the final preparation of this report.

No qualitative or quantitative information specific to the toxaphene content of Canadian drinking water supplies were found in the literature.

However, this author is of the opinion that the Canadian total diet monitoring programme carried out by Health & Welfare Canada from 1969 to 1975 (83, 84, 85, 86) provided presumptive evidence for the absence of toxaphene in potable water. Soft drinks, containing in excess of 90% water, in many instances obtained from local water supplies, were analyzed and found to be free of toxaphene. The analytical methods used were capable of det detecting toxaphene at less than 10 ug/l concentrations.

5.1.2 Food

There have been many analyses of toxaphene in treated crops (69). Generally, these showed residues were likely to occur with a specific pesticide treatment, or multiple treatments, used during the production of the crop. Table 1 summarizes selective data which indicate the levels of residues that resulted from usual

Table 1 TOXAPHENE RESIDUES RESULTING FROM SUPERVISED TRIALS

Crop	Rate of Application (kg/ha)	No. of Treatments	Pre-harvest* Interval (days)	Residue at Harvest (ppm)	Comments
Vegetables					
Lettuce	5.5	1 - 4	10	5.8 - 7.9	Whole head
Kale	5.0	4	36	3.3 - 7.2	
Cabbage	1.9 - 12	2 - 6	9 - 38	0.8 - 6.6	On outer leaves
Spinach	5.0	4	30	16.7 - 18.8	
Celery	1.1 - 1.6	9	13	1.8 stalks 6.5 leaves	Washed
Cauliflower	3.8	1	8	1.1	Processed commercially & frozen before analysis
Broccoli	10	1	8	3.4	
Tomatoes	1.3 - 2.5	8 - 9	5 - 7	2.0 - 4.3	
Greenbeans	7.5	1	7	1.3	Unwashed
Lima beans	3.9	1	14	0.3	Shelled beans
Carrots	25	2 - 4		0.9 - 3.3	Soil appl.(1 yr)
Potatoes	0.95 - 2.5	6	21	0 detected	
Field peas	2.5	3	4	1.8	
Oil Seeds					
Cotton (seed)	3.9 - 5.0	15	6	3.6 - 5.2	Lint bearing seed
Soybeans	3.8	3	60	0.5	
Peanuts(shelled)	25 - 50	1		0 detected	Soil treat.
Fruit					
Oranges	5.7	2	7 - 70	0 - 10.9	Skins
				0 - 0.3	Pulp
Bananas	3.8	1	1	0.3 - 1.3	Whole fruit
Pineapple	2.8	2	84 - 96	1.3 - 2.7	Whole fruit
Cereal Grains					
Wheat	1.9 - 3.8	1	14 - 21	0.5 - 1.8	
Barley	1.9 - 3.8	1	7 - 28	0.7 - 14.2	
Oats	1.9 - 3.8	1	7	1.0 - 2.6	
Rice	1.9 - 3.8	1	7 - 28	1.5 - 5.6	Unfinished grain
Sorghum	2.5	1	28	2.5 - 3.1	
Corn (maize)	2.5	1	12	0.08	Kernels
Fat of Meat Animals					
Beef	0.5%	12	28	5.0	12 weekly sprays
Swine	0.5%	2	28	0 - 0.6	2 sprays
Shelled Nuts					
Almonds	4.0	3	135	1.5	

(*) Interval from last application if multiple applications were made.

agricultural practice on representative types of crops. The residue level is controlled by proper application restrictions; such as pre-harvest intervals, application prior to formation of edible parts or application to crops where the harvested portion is protected from the spray by pods, husks, outer leaves or other coverings.

Toxaphene residues resulting from a foliar application generally exhibit a half-life of 5 - 10 days on growing crops. The half-life varies with weathering conditions, plant, or fruit type, plant growth rate, and for different formulations. Residues from wettable powders or dusts are lost more rapidly than from emulsifiable concentrates. For example, on snap beans, with no rain, a steady decline from 8.1 mg/kg to 1.5 mg/kg occurred in 12 days. Rain markedly hastened the disappearance of residues in other crops. Tomatoes, with a small surface area in comparison to total volume, had a residue which declined from 4 mg/kg to 0.15 mg/kg in 22 days. In contrast, collards, with a large surface area in relation to total volume, had a residue which decreased from 168 mg/kg to 5.0 mg/kg in 12 days.

The available data indicated that there is a very low incidence of toxaphene in the Canadian food supply. In the total diet pesticide residue programme carried out by Health & Welfare Canada from 1969 to 1973 (21, 85, 86), no evidence of detectable levels of toxaphene were found in any of several hundred food samples analyzed, even though the methods used were adequate for its detection at the ug/kg (ppb) level.

In the total diet sampling programme carried out during 1974-1975, which was the last active year for the monitoring programme, the incidence of toxaphene residues in food items was reported as being 0.6%. This survey included food from 4 different cities across Canada, which made it a more comprehensive monitor of food levels than had been done in previous years in which food from only 1 location was analyzed (83).

In the author's opinion it is significant that the Health & Welfare report (83) failed to provide quantitative data or information as to the type of food item(s) which contained toxaphene. This suggests that there is no great concern over the toxaphene content of the Canadian food supply.

There is no convincing evidence to indicate an excessive build-up in the soil from the normal use of toxaphene, or that residues in the soil are absorbed by plants and concentrated to any degree. Because foliar application is the major use of toxaphene, residues in the soil would derive primarily from this source.

Cows fed or sprayed with toxaphene produced milk which contained the pesticide (87). Studies indicated that the ratio of toxaphene in the milk to that in the feed was about 1:100. Uncontaminated milk was produced within 14 days after discontinuing feed containing up to 10 mg/kg. Dermal application of toxaphene has never been permitted on dairy animals (69).

Toxaphene was not among the 15 pesticide chemicals most frequently found in the 888 total diet composites examined from 1964-1967 in the U.S.A. (11). When toxaphene was found in the total diet composites, it was usually in either the leafy vegetable or garden fruit composite samples. For the period June 1964 to April 1967, toxaphene was found in 1.4% of the leafy vegetable composites at an average level of 0.005 mg/kg (ppm) and in 2.7% of the garden fruit composites at an average level of less than 0.001 mg/kg.

From 1963-1966, 26,326 U.S.A. raw agricultural samples (not including animal tissues) were found to contain pesticide residues. Toxaphene was present in 754 of these, and ranked 10th in incidence among the 11 pesticides which contributed to 95% of all residues found during that period. Toxaphene ranked third in incidence in animal tissues in the U.S.A.

Table 2 shows the percentage of U.S. samples from 1964-1967 which contained toxaphene and the average level found for various raw agricultural products.

Among the samples of canned and frozen products examined from 1964-1967 in the U.S.A., toxaphene ranked 6th in incidence, with 5% of all the samples containing residues at an average level of 0.45 mg/kg, which was the highest average level found for the 17 most frequently encountered pesticide residues (11, 69).

It was notable that the edible flesh of Bluegill contained only 10% of the body residue of toxaphene (43) and the effect of cooking on fish residue levels was somewhat variable. With the bluegill, 9-27% of the toxaphene residue was removed on frying (43), whereas with rainbow trout there was no reduction in the toxaphene level after 10 minutes of frying at 375°F, or boiling in water for 15 minutes (30).

It is noteworthy that rainbow trout, from water containing 0.41 ug/l (ppb) of toxaphene, contained 7.72 mg/kg of toxaphene (whole body). Atlantic salmon, which came from water containing 0.4 to 0.66 ug/l, contained 5.5 mg/kg of toxaphene, based on whole body analysis (75).

5.1.3 Air

Stanley and co-workers (39) investigated the atmospheric levels of pesticides at 9 localities in the United States and detected pesticides at levels ranging from the lower limit of detection of 0.1 ng/m^3 of air to as high as 2520 ng/m^3. Only DDT was detected at all localities. Toxaphene was found to be present in air samples taken in Stoneville, Mississippi, at a concentration between 81 and 1110 ng/m^3; as well as in the air at Dothan, Alabama (68 ng/m^3), and Orlando, Florida (2520 ng/m^3). The highest toxaphene levels at Stoneville were reported between August 14 and October 2, and again between July 1 and August 13. Although the pesticide levels varied from day to day and with the

Table 2

INCIDENCE AND CONTENT OF TOXAPHENE IN AGRICULTURAL PRODUCTS IN THE U.S.A. FROM 1964 - 1967

Raw Agricultural Product	Incidence Percent	Average ppm
Leaf and stem vegetables	6.4	0.18
Vine and ear vegetables	1.4	0.01
Root vegetables	1.1	< 0.005
Small fruit	1.0	< 0.005
Beans	0.9	< 0.005
Large fruit	0.3	< 0.005
Cereal grains	0.3	< 0.005
Nuts	0.3	< 0.005
Eggs	0.2	< 0.005
Animal food grains	0.1	< 0.005

season, the highest levels were generally found during periods of application in the area. Whether a seeming decrease of pesticide levels with rainfall was real or was a result of the fact that no spraying was carried out on a rainy day was not determined. However, it appeared that if several days of rain had taken place, then pesticide levels usually decreased. The highest pesticide concentrations were found in the agricultural areas of the south. Relatively low levels (no toxaphene detected) were reported in other agricultural areas, such as Buffalo and Iowa City. Appreciable levels of pesticides, but not toxaphene, were found in Salt Lake City, an urban area in which there was considerable mosquito control activity. Urban areas, such as Baltimore, Fresno and Riverside contained no detectable levels of toxaphene.

The atmospheric level of various pesticides, including toxaphene, was investigated by Arthur et al. (88), in the mid-Mississippi delta region. This area was considered one of the highest pesticide usage areas in the United States because of its intensive cotton production. The investigators took weekly air samples during 1972, 1973 and 1974 and analyzed them for pesticide content. The maximum level of toxaphene found in the weekly air sample was about 1750 ng/m^3. Except for methyl parathion, toxaphene concentrations were the highest of the pesticides studied. The average monthly atmospheric levels of toxaphene showed that the highest concentrations were found in July, August and September. The average levels for 1973 were about 1/3 of those for 1972 and were attributed to a 55% decrease in the use of toxaphene from one year to the next. In 1974, the use of toxaphene increased 22% over the previous year and the levels detected were almost double those of 1973. The average value seen in 1974 was 150 ng/m^3, with the highest average monthly level (903 ng/m^3) being reported in August. Between December and March the level of toxaphene fell to between 10 and 20 ng/m^3.

No information on toxaphene levels in Canadian air were available in the literature reviewed. In view of the fact that the pesticide is not in general use in Canada and was only found in the U.S.A. atmosphere in locations of extensive use, it can be reasonably assumed that the Canadian air would be free of toxaphene.

5.1.4 Soil And Sediment

Soil surveys in the U.S.A. have shown toxaphene to be a contaminant in soil at a mean residue level of 0.07 mg/kg (range 0.10-11.72) in 4.2% of the samples analyzed (77).

Carey et al. (47) surveyed 14 cities in the United States in 1970 for soil residue of various pesticides. Toxaphene was found in only 3 of 28 sites sampled in Greenville, Mississippi, where the level of residues ranged from 7.7 to 33 mg/kg. The data suggested that contamination of soil in cities from toxaphene was not a serious problem and could only be found in areas where toxaphene was being used (eg. the cotton producing south eastern states).

The fate of DDT and toxaphene applied in combination on cotton in a 3-year rotation with oats, clover and grain sorghum was studied by Swoboda et al.,(48). The soil was a heavy clay situated on three black-land prairie watersheds in Texas. Between 1957 and 1963 the watersheds received approximately 10, 18, or 22 pounds of toxaphene per acre. The authors concluded that the amount of toxaphene found in the three watersheds was relatively small in comparison to the amount applied. The field which received the lower amount of toxaphene had a significantly lower contamination of toxaphene than the other fields, indicating that the quantity recovered was related to the amount applied. A higher percentage of the applied insecticide was recovered in the field receiving the lower rate of toxaphene, than in the two fields receiving the higher rates. The relative distribution suggested that more recently applied toxaphene was closer to the surface, however, unlike DDT, there was no appreciable toxaphene at depths greater than 1 foot. It was estimated that 90% or more of the recoverable toxaphene was in the top foot of soil. The complete lack of toxaphene in drainage water taken from a nearby seep also suggested that toxaphene did not move very deep. The authors noted that toxaphene could be expected to be lost due to spray drift, as well as on harvested portions of plants and by volatilization from the soil. Since the soil surface contained most of the pesticides and the Houston black clay reached temperatures of 140°F almost daily in the summer it seemed reasonable that large losses could occur by volatilization. It was estimated that 70-80% of the toxaphene was lost over the 11-year period and this was higher than reported in other climatic regions.

During surveys of the hydrocarbon content of the lower Mississippi River, toxaphene was found in only a few instances and then at trace levels (66).

The sediment of a stream into which a toxaphene plant outfall emptied was found by Durant and Reimold (58) to contain toxaphene up to a concentration of about 2000 mg/kg (ppm).

Possible reintroduction of toxaphene into estuarine biota from dredging and displacement of contaminated sediment was studied in a creek in Georgia (74). The mean of the weekly concentration of toxaphene in oysters in May was 3.3 mg/kg, whereas the oysters collected a month later on the day the dredging operation began, had a higher concentration than subsequent samples but contained 1.24 mg/kg less than the May average. Analyses of toxaphene in the sediment core showed the concentrations in the first 10 centimetres to range from 35 to over 1,850 mg/kg. The composite sample of surface sediment obtained after dredging contained about 33 mg/kg of toxaphene on a dry-weight basis.

The authors suggested that the expected fish kills during dredging did not occur because toxaphene was bound to clay particles. They also suggested that there was significant degradation of toxaphene below the 10 centimetre sediment depth.

5.1.5 Aquatic Biota

A study of the distribution of various insecticides, including toxaphene, in a large lake in the predominantly agricultural region of South Dakota,was carried out by Hannon and colleagues (44). The methodology employed had minimum limits of 20 ug/kg in fish, 5 ug/kg in plankton-algae, 1 ug/kg in water and 10 ug/kg in bottom sediment. Toxaphene was found to be present in only 4 of 87 fish samples analyzed, with levels ranging from 0.09 to 1.05 mg/kg. All other samples including water, sediment, crayfish, aquatic insects and plankton had less than detectable amounts of toxaphene. Residues of other insecticides such as lindane, DDT, heptachlor and aldrin were almost universally present.

Butler (42) reported on the organochlorine residues in various estuarine mollusks such as clams and oysters. Fifteen coastal states were monitored during 1965-1972 with toxaphene residues being found in mollusks from only California, Georgia, and Texas. In Georgia,128 of 664 samples contained toxaphene whereas in California, 4 of 772 were contaminated, and only 1 of 728 in Texas had detectable amounts. It is interesting to note that the maximum residue detected was 54,000 ug/kg (ppb).

The chlorinated hydrocarbon levels in livers of fishes from the northeastern Pacific Ocean (California, Washington and Alaska) were measured by Duke and Wilson (41). No toxaphene was detected in any of the specimens analyzed, whereas DDT, DDD and, in some instances, PCB's were found.

Fresh water plankton were found to contain 317 ug/g (ppm) of toxaphene, whereas various fish from the same lake had levels of 4 ug/g (26).

Mehrle and Mayer (89) studied toxaphene residues in the fathead minnow. They found fish exposed to 55 - 132 ng/l (ppt) contained 0.5 to 48 ug/g (ppm) of toxaphene, which was the residue range reported for fish by the U.S. National Pesticide Monitoring Program in 1974.

6. EXPERIMENTAL TOXICOLOGY

6.1 Metabolism

The study of the absorption, distribution and metabolism of toxaphene is exceedingly difficult due to a lack of suitable microanalytical methods for the detection of low residue levels in tissues, which is further complicated by the chemical complexity of the product, in that it is a mixture of over 175 C_{10} polychlorinated derivatives.

The route and rates of excretion of toxaphene from rats has been studied to a limited extent using radiotracer techniques.

For example, Crowder and Dindal (90) administered 20 mg/kg of ^{36}Cl-toxaphene to rats and found that 53% of the oral dose was excreted within 9 days, with feces containing about 37% and urine 15% of the excreted radioactivity.

The most extensive work has been carried out at the University of California (Berkley) by the group of Casida.

In 1974 they reported the metabolism of orally administered ^{36}Cl-toxaphene (6) at a dose of 14 mg/kg. Most of the labelled compound was metabolized before excretion, with less than 0.7% of the ^{36}Cl-toxaphene appearing in the urine and less than 3% in the feces as unmetabolized toxaphene. About 5% of the administered ^{36}Cl-toxaphene appeared in urine and 21-24% in feces as partially dechlorinated metabolites. The remainder of the excreted ^{36}Cl-toxaphene, accounting for 44-57% of the administered labelled toxaphene dose, was chloride ion in the urine.

For comparison, 90% of $Na^{36}Cl$ administered to control rats was excreted as urinary chloride ion.

During these investigations 7 sub-fractions of toxaphene, as well as toxaphene itself, were studied. The findings suggested that most of these components contained the same or similar biodegradable groupings and all undergo in vivo dechlorination in mammals. It was calculated that about 50% of the C-Cl bonds were metabolically active.

The same group reported the results of further investigations (19). Toxaphene, labelled with ^{36}Cl or ^{14}C was administered orally to rats with 50-60% of the ^{36}Cl being excreted in the urine and 30-40% in the feces within 14 days. These authors pointed out that the metabolism of toxaphene was difficult to study because of the presence of more than 177 C_{10} polychloro compounds, of which only one (Toxicant B - 2,5-endo,6-exo,8,9,10-heptachlorobornane) had been identified. Technical ^{36}Cl-toxaphene was administered to 250-290 g rats at about 13 mg/kg and ^{14}C-toxaphene at 8.5 and 19.0 mg/kg. Toxicant A and toxicant B, labelled with ^{14}C, were administered at 0.84 and 2.6 mg/kg respectively to 200-225 g rats. Seven other labelled fractions were also given to these animals with each probably containing some 26-47 compounds, which made the interpretation of the data complex. The investigators pointed out that toxaphene and the seven fractions underwent essentially the same extent of dechlorination, with about half of the C-Cl bonds being cleaved prior to excretion.

Although there were large toxicity differences among the toxaphene fractions, this was not reflected in their extent of metabolism. Tissues were found to retain relatively low levels of ^{14}C for several days after the administration of toxaphene or toxicant B. Of the total radioactive compound administered, about 60% of the dose was recovered, with 35% being in the feces and 20% in urine. The expiration of $^{14}CO_2$ accounted for only about 1% of the administered dose.

These experiments showed that toxaphene underwent extensive dechlorination and/or dehydrochlorination in vivo.

Figure 4, taken from the publication of Ohsawa et al.,(19), summarizes the excretion-time patterns for ^{36}Cl and ^{14}C-toxaphene.

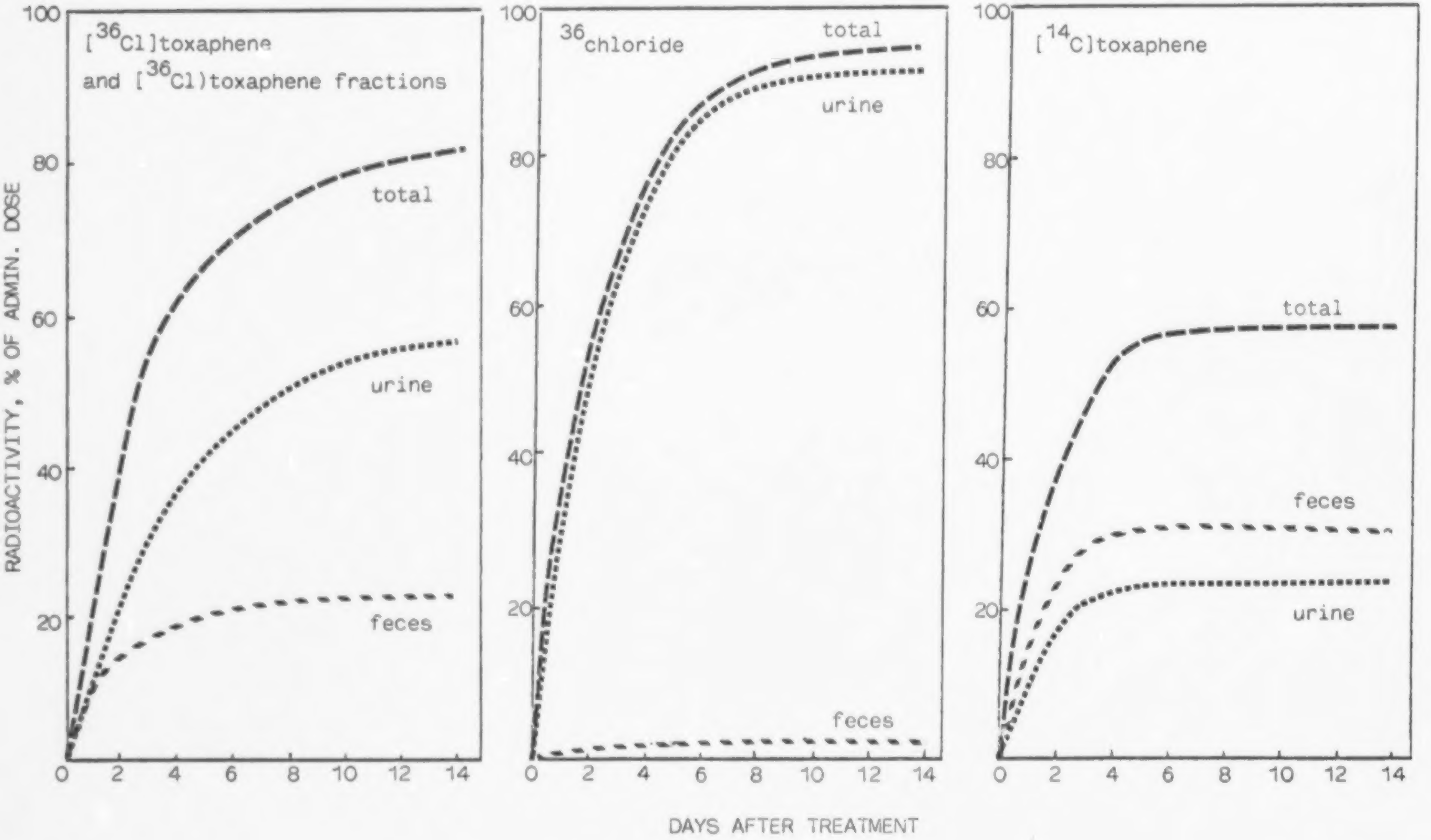
The distribution of radiocarbon in rat tissues was found to be equivalent to 0.78 mg/kg (ppm) in fat and 0.3 mg/kg in liver 14 days after a 19 mg/kg dose of ^{14}C-toxaphene. An 8.5 mg/kg dose resulted in 0.52 mg/kg in fat, 0.12 mg/kg in liver, 0.17 mg/kg in kidney, 0.14 mg/kg in blood and 0.02-0.09 mg/kg in each of bone, brain, heart, lung, muscle, spleen and testes 14 days later.

Nine days after a 2.6 mg/kg dose of ^{14}C-toxaphene, toxicant B levels in liver were 0.12 mg/kg, in kidney 0.09 mg/kg, in blood 0.07 mg/kg and less than 0.03 mg/kg in other tissues (fat was not analyzed). The data indicated that toxaphene and toxicant B were not persistent in rats after oral treatment.

Khalifa et al. (13) also reported that toxaphene, toxicant A and toxicant B were metabolized extensively by isolated rat liver microsomes in an in vitro system which required both NADPH and anaerobic conditions, thereby suggesting that reduced cytochrome P-450 acted as a reducing agent. Their study further established that toxaphene underwent extensive dechlorination and that the products from toxicants A and B were formed by 2 or more of the following processes: reductive dechlorination, dehydrochlorination, and vicinal chloride elimination.

Little information was in the literature on the metabolism of toxaphene in fish, a species which is particularly sensitive to the pesticide. Although Schaper and Crowder (91) studied the uptake of ^{36}Cl-toxaphene by mosquitoe fish, their data only examined its uptake and distribution. Ninety percent of the mean fatal dose of toxaphene had been adsorbed by the time the fish exhibited their first signs of toxicity. Excretion was

FIGURE 4. Excretion of radioactive products by rats treated orally with [^{36}Cl]toxaphene, [^{36}Cl]toxaphene fractions, sodium [^{36}Cl]chloride, and [^{14}C]toxaphene. (From Reference 19)



not observed following an 8-hour exposure to 2 mg/l and metabolic alteration of toxaphene appeared to be minimal.

6.2 Acute Oral And Parenteral Toxicity

During the extensive review of the literature of toxaphene many papers on the toxicity of the compound were examined. Several of the studies used doses of the pesticide which were sufficiently high as to be overtly toxic. These data were not particularly useful in assessing the toxicity of toxaphene at low concentrations in water, or on chronic administration as would be most relevant to the toxicity of toxaphene in drinking water.

Tables 3 - 11 provide summaries of the toxicity studies reviewed and include the species used, route and duration of administration as well as a summary of the observed effects. The data have been further delineated into acute (Tables 3-9), sub-acute (Table 10) and chronic (Table 11) investigations.

Only selective reports are discussed in the text, with emphasis being given to those providing information on the toxicity of toxaphene at low doses and for prolonged periods of time. When referring to the Tables in the text, the second number indicates the sequence of the summary in the Table.

6.2.1 Mammals

Summaries of acute toxicity data in mammals can be found in Tables 3 and 4.

6.2.1.1 Dogs

Soon after its initial use by the U.S. Army, during the latter stages of the second world war, reports began appearing in the literature which described the acute toxicity of toxaphene to a variety of animal species.

Table 3: Summary Of Mammalian Acute Toxicity Studies

SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
1. Dog	5 mg/kg	Oral	Single	- (in corn oil) no convulsions or deaths	92 (1949)
	10 mg/kg	Oral	Single	- (in corn oil) caused convulsions but no deaths	
	15 mg/kg	Oral	Single	- 6 of 8 animals had convulsive seizures; 2 died	
	25 mg/kg	Oral	Single	- approximate LD_{50} (in corn oil)	
	200 or 250 mg/kg	Oral	Single	- in kerosene solution; 8/10 convulsed, one died	
	400 mg/kg	Oral	Single	- approximate LD_{50} (in kerosene)	
	4 mg/kg	Oral	Daily for 106 days	- gross pathologic changes not observed, but degenerative changes in liver parenchyma and renal tubules evident	
2. White Rat	—	Oral	Single	- acute oral LD_{50} = 90 mg/kg	93 (1959)
3. Rat	—	Oral	Single	- acute oral LD_1 = 15 mg/kg (male); 44 mg/kg (female)	94 (1969)
4. White Rat	--	Oral	Single	- acute oral LD_{50} = 90 mg/kg (male)	94 (1969)
				- acute oral LD_{50} = 80 mg/kg (female)	11, (1975)
5. White Rat	1% in peanut oil & acacia	Oral	Single	- acute oral LD_{50} = 45 mg/kg	95 (1975)
	20% in kerosene	Oral	Single	- acute oral LD_{50} between 100 - 150 mg/kg	
	5% in kerosene	Oral	Single	- acute oral LD_{50} between 300 - 350 mg/kg	
	5% in corn oil	Oral	Single	- acute oral LD_{50} between 120 - 125 mg/kg	
	1% in peanut oil & acacia	Intravenous	Single	- acute i.v. LD_{50} = 13 mg/kg	
6. Mouse	42 mg/kg	Intraperitoneal	Single	- LD_{50} toxaphene	15 (1974)
	3.1 mg/kg	Intraperitoneal	Single	- LD_{50} toxicant A	
	6.6 mg/kg	Intraperitoneal	Single	- LD_{50} toxicant B	

Table 3: continued

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
7.	Mouse	42 mg/kg	Intraperitoneal	Single	- LD_{50} toxaphene	19 (1975)
8.	Rat	20 ml solution in cottonseed oil/kg	Oral	Single	- diet protein deficient LD_{50} = 80 ± 19 mg/kg - normal protein diet LD_{50} = 293 ± 31 mg/kg - commercial diet LD_{50} = 220 ± 33 mg/kg	96 (1975)
9.	Wild Mice	Not given	—	—	- wild mice more resistant to toxaphene than Swiss-Webster Strain (abstract)	97 (1970)
10.	Mouse	42 mg/kg	Intraperitoneal	Single	- LD_{50} = 42 mg/kg	5 (1975)
11.	Rat	— —	Oral Oral	Single Single	- acute oral LD_{50} = 69 mg/kg (Metcalf in Ref. 98) - acute oral LD_{50} = 90 mg/kg (Henderson & Pickering in Ref. 98)	98 (1967)
12.	Goat rumen microorganisms	10, 100 & 1000 ppm	*In Vitro*	—	- 10 and 100 ppm showed only a small effect on digestion of dry matter and plant cell wall material - at 1000 ppm both types of digestion were inhibited significantly - exposure of animals to this amount of toxaphene in their diet would exceed the LD_{50}	99 (1973)
13.	Deer rumen microorganisms	10, 100 & 1000 ppm	*In Vitro*	—	- 10 and 100 ppm - no effect on dry matter digestion - 1000 ppm - digestion reduced to 65 - 70% of untreated controls - to reach these conc. in animal rumen, estimated LD_{50} would be exceeded	100 (1974)
14.	Adult cattle Dairy calves	35 mg/kg 5 - 10 mg/kg	Oral Oral	Single Single	- minimum toxic dose - minimum toxic dose to animals under 2 weeks of age	101 (1970)
15.	Sheep, Angora Goats	25 mg/kg	Oral	Single	- minimum toxic dose to adult animals	101 (1970)

Table 4 LD$_{50}$ VALUES FOR TOXAPHENE TO SEVERAL MAMMALS (From Ref. 102 & 69)

Species	Route	LD50 (mg/kg)	Vehicle*
Rat	Oral	90	Peanut oil
Rat	Oral	60	Corn oil
Rat	Oral	120	Kerosene
Mouse	Oral	112	Corn oil
Dog	Oral	49	Corn oil
Dog	Oral	> 250	Kerosene
Guinea pig	Oral	270	Corn oil
Guinea pig	Oral	365	Kerosene
Cat	Oral	25-40	Peanut oil
Rabbit	Oral	75-100	Peanut oil
Rabbit	Oral	250-500	Kerosene
Cattle	Oral	144	Grain
Goat	Oral	200	Xylene
Sheep	Oral	200	Xylene
Rat	Dermal	930	Xylene
Rabbit	Dermal	> 4,000	Dust
Rabbit	Dermal	< 250	Peanut oil

*The vehicle used to apply a toxicant may influence the rate and extent of toxicant absorption by the animal, whether by oral or dermal or other mode of application. Furthermore, some vehicles may themselves be significantly toxic.

For example, in 1949 Lackey (92)(Table 3-1) reported the acute and chronic toxicity of toxaphene in the dog. The insecticide was administered orally in either corn oil or in kerosene solution. At a dose of 10 mg/kg in corn oil, 4 of 5 dogs had convulsions, but none died. At 15 mg/kg, 6 out of 8 dogs convulsed and 2 of the animals died. The LD_{50} was determined to be near 25 mg/kg. For toxaphene in kerosene, a dose of 75 mg/kg resulted in one animal convulsing. At 200 and 250 mg/kg, 4 of 5 dogs convulsed, and 1 in each group died. The LD_{50} for toxaphene in kerosene was calculated to exceed 400 mg/kg, showing that the solvent greatly decreased the absorption of the insecticide, compared to a digestible oil such as corn oil.

It was later shown (1951) that a dose of toxaphene of 15 mg/kg could result in convulsions and death in the dog. In a study designed to investigate the treatment of convulsions occurring during toxaphene toxicity, Lackey and Weed (127) gave 50 mg/kg orally to a group of dogs. Treatment was inhibited at the onset of convulsions, or in some instances, before the expected convulsions began. The investigators reported that when treatment was instituted before the onset of convulsions, phenobarbital was the most effective drug. When convulsions had already developed, their information suggested that pentobarbital could be used more effectively.

6.2.1.2 Rodents

Several reports of the acute toxicity of toxaphene in mice and rats indicate it to be quite variable.

For example, Henderson et al. (93)(Table 3-2) found the oral LD_{50} in the white rat to be 90 mg/kg of body-weight, as have others (11, 94)(Table 3-3 & 4) who published values of 90 mg/kg for male and 80 mg/kg for female rats.

In contrast, McGee et al. (95)(Table 3-5) reported that in white rats the oral LD_{50} for toxaphene in a 1% emulsion of peanut oil and acacia to be 45 mg/kg. The LD_{50} for a 20% solution in kerosene was 100-150 mg/kg, and for a 5% kerosene formulation, 300-350 mg/kg. Five percent toxaphene in corn oil had an LD_{50} of 120-125 mg/kg, indicating that the vehicle had a large affect on the toxicity of the pesticide.

This same group (95) determined the intravenous LD_{50} of a 1% toxaphene emulsion in peanut oil and acacia to be approximately 13 mg/kg.

The LD_1 for oral toxaphene in the rat was 15 mg/kg for males and 44 mg/kg for females (94).

Khalifa et al. (15) and Ohsawa et al. (19)(Table 3- 6 & 7) also examined the toxicity of several toxaphene fractions, including toxicants A and B in the rat and mouse. Toxaphene itself had an i.p. LD_{50} in mice of 42 mg/kg, whereas some of the other fractions had toxicities which varied from 16 to 252 mg/kg, showing that there were large differences in the toxicities of the various components.

Toxicant A had an i.p. LD_{50} in the mouse of 3.1 mg/kg and toxicant B, 6.6 mg/kg (15)(Table 3-6). Toxicant A was found to make up about 6% and toxicant B 3%, of technical toxaphene. Therefore, these two components could contribute significantly to the toxicity of the pesticide. However, it is likely that there are other highly toxic components present as well.

Boyd and Taylor (96)(Table 3-8) investigated the effects of diet on the acute toxicity of toxaphene in rats. Animals were fed either a low protein diet (3.5% casein), a protein supplemented diet (26% casein), or standard laboratory rat chow. Their findings are summarized in Table 5 and indicate that a low protein diet increases the toxicity of the pesticide. The incidence of effects observed at autopsy were: encephalitis, 90-100%; pale splein, 40%; pneumonitis, 30-70%; enteritis, 20-30% and congested adrenal glands, 5-40%. In general, the pesticide

Table 5 THE EFFECT OF DIET ON THE ACUTE TOXICITY OF TOXAPHENE (From Reference 96)

	Low Protein Diet	Protein Supp. Diet	Lab Chow Diet
Estimated LD_1 (mg/kg)	1.1	191	139
LD_{50} (mg/kg)	80	293	300
Hours to Death	39	25	24

produced clinical signs of depression and stimulation of the central nervous system, anorexia, oligodipsia, loss of body weight, diuresis, glycosuria and hypothermia.

6.2.1.3 Others

In guinea pigs the LD_{50} by oral administration of 5% toxaphene in kerosene was 350-375 mg/kg and in 5% corn oil, 250-280 mg/kg (95) (Table 3-5).

Luckens (97)(Table 3-9), in an abstract of a presentation, pointed out that native wild mice were more resistant to the toxic effects of toxaphene than the Swiss-Webster strain of white mice.

6.2.2 Birds

The toxicity of toxaphene has been determined for a number of birds. Acute oral toxicity values (LD_{50}) were given as follows: young pheasants, 40.0 mg/kg; sharp-tailed grouse, 10-20 mg/kg; young mallards, 70.7 mg/kg; young bobtail quail, 85.4 mg/kg; lesser sandhill cranes, 100.0-316.0 mg/kg; fulvous tree ducks, 99.0 mg/kg,(53)(Table 6-1).

Birds, as a group, do not appear to be resistant to toxaphene, having a toxicity range comparable to that of smaller laboratory

Table 6: Summary Of Bird Acute Toxicity Studies

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
1.	Various birds	—	Oral	Single	- acute oral LD_{50}: young pheasants, 40.0 mg/kg; sharp-tailed grouse, 10 - 20 mg/kg; young mallards, 70.7 mg/kg; young bobtail quail, 65.4 mg/kg; lesser sandhill cranes, 100.0 - 316.0 mg/kg; fulvous tree ducks, 99.0 mg/kg	53 (1977)
2.	Birds	—	—	—	- do not appear to be resistant to toxaphene - toxicity range comparable to that of small laboratory animals (50 - 200 mg/kg body weight)	49 (1953)

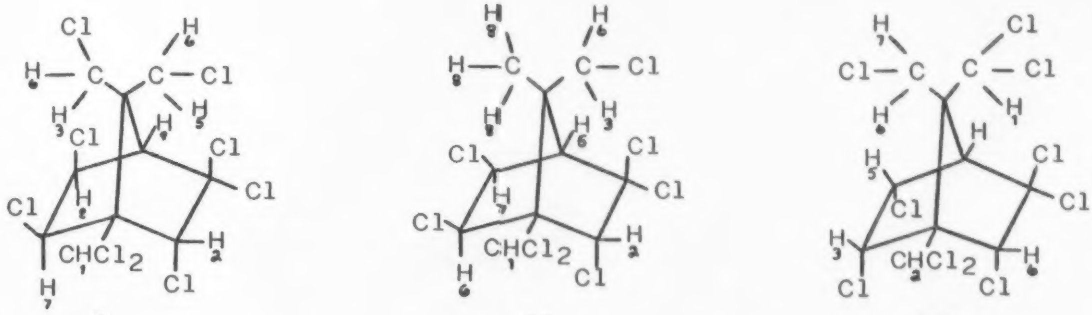
animals (50-200 mg/kg of body weight),(49)(Table 6-2).

6.2.3 Insects

Water treatment with toxaphene for fish eradication also appears to have a marked effect on lake invertebrates, particularly insect larvae. Microcrustacea in general appeared to be less sensitive than insect larvae. Some larvae have a 24-hour TLM value up to 75 times greater than that of the target species of fish, such as the blue-nosed minnow, which competes with trout for food. However, no matter how severe the effect of toxaphene on the different non-target fauna, repopulation usually occurs sooner or later and conditions have been reported as normal within 1 year of treatment (80)(Table 7-1).

Three components of toxaphene were tested for their toxicity to the common housefly and compared with that of the standard toxaphene mixture (14)(Table 7-2). It was found that components I, II and III (see Figure 5 for structures) had toxicities 2 to 4 times that of the toxaphene mixture itself, with compound I having an LD_{50} of 0.5, ug/fly; II, 0.91; and III, 1.2, whereas toxaphene had an LD_{50} of 2.1.

FIGURE 5. THE STRUCTURE OF THREE TOXIC COMPONENTS OF TECHNICAL TOXAPHENE (From Reference 14)



I II III

Table 7: Summary Of Insect Acute Toxicity Studies.

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
1.	Insect larvae (various spp.)	0.02 - 0.1 ppm	Immersion	—	- insect larvae more affected than micro-crustaceae - some insect larvae have a 24-hour TL_m value several (up to 75) times greater than common target fish species - re-population of non-target species is usually complete in a year	80 (1971)
2.	Housefly	2.1 ug per insect	Topical	—	- LD_{50} toxaphene - also found that 3 toxaphene fractions had toxicities 2 to 4 times that of toxaphene itself (0.5 to 1.2 ug/insect)	14 (1974)
3.	Housefly	—	Topical	Single	- topical LD_{50} = 31 ug/g	9 (1976)
4.	Housefly	31 mg/kg	Topical	—	- LD_{50} toxaphene (Metcalfe in Ref. 98)	98 (1967)
5.	Housefly (*Musca domestica L.*)	70 mg/kg	Topical	24 hours	- LD_{50} toxaphene	5 (1975)
6.	Housefly (*Musca domestica L.*)	70 mg/kg	Topical	24 hours	- LD_{50} toxaphene - the LD_{50} range for 7 different fractions of toxaphene was from 42 to 316 mg/kg	19 (1975)
7.	Housefly (*Musca domestica*, UCR Susc)	33 mg/kg	Topical	24 hours	- LD_{50} camphene component* = LD_{50} toxaphene [*(2,5,6-exo,8,8,9,10-heptachlorodihydrocamphene)]	9 (1976)
8.	Housefly (*Musca domestica L.*, SCR Susc. Strain)	70 mg/kg	Topical	24 hours	- LD_{50} toxaphene (toxaphene and its fractions were dissolved in acetone)	15 (1974)
		16 mg/kg	Topical	24 hours	- LD_{50} toxicant A	
		32 mg/kg	Topical	24 hours	- LD_{50} toxicant B	
9.	Housefly	—	Topical	Single	- topical LD_{50} = 31 ug/g	93 (1959)

Table 7: continued

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
10.	Lady Beetle (Coleomegilla maculata)	10 ug	Topical	10 days	- LD_{50} = 21.8 ug (1789 mg/kg)	103 (1966)
		10 ug	Topical	10 days	- diapause LD_{50} = 44.2 ug (3619 mg/kg) - toxaphene dissolved in acetone - toxaphene decreased longitivity and oviposition	
11.	Stonefly niads (Pteronarcys californica)	18 ug/l	Immersion	24 hours	- LC_{50}* (24 hour) (* - median lethal concentration)	107 (1968)
		7 ug/l	Immersion	48 hours	- LC_{50} (48 hour)	
		2.3 ug/l	Immersion	96 hours	- LC_{50} (96 hour) - the 96-hour LC_{50} for all three species tested ranged from 1.3 - 3.0 ug/l	
12.	European Corn Borer	1 - 2 lbs/ day	Granular in corn plots	Single	- effective control throughout 8-day evaluation - in general, granular formation of toxaphene more effective than spray	128 (1976)
13.	Mosquito larvae Brine shrimp, algae	—	Immersion	24 hours	- LC_{50} toxic fraction A/LC_{50} toxaphene = 1.87, 1.75, 1.35 for mosquito larvae, brine shrimp and blue green algae, respectively	18 (1975)
14.	Tobacco budworm (Heliothis virescens)	3.52 mg/g	Topical	48 hours	- LD_{50} of toxaphene:DDT (2:1) in acetone - resistance to the above mixture developed 6-fold since 1963 in these larvae	105 (1967)
15.	Tobacco budworm	5 mg/g	Topical	—	- LD_{50} toxaphene	104 (1973)
	Bollworm (Heliothis Zea, Boddie)	200 ug per larvae	Topical	—	- not lethal to these larvae	
16.	Tobacco budworm	5.5 mg/g	Topical	—	- LD_{50} toxaphene	106 (1969)
17.	Fabric Pests	0.8% of fabric weight	Topical	—	- prevented damage by clothes moth, black carpet beetle, furniture beetle	2 (1974)

Table 7: continued

SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
Lepidopterous larvae	0.1%	Topical Spray	—	- effective control against tortricid caterpillars on fruit	2 (1974)
Various Insects	0.2%	Topical Spray	—	- effective control of psyched moth (bagworm), grasshoppers (Melanoplus spp.), pentatomid and mirid plant bugs, trips on cotton, various boring larvae, and weevils - combined stomach and contact poisoning action yield effective control - in agricultural situations prior to 1955 were adequately controlled by toxaphene. Now used 2:1 (toxaphene:DDT) at 1 - 6 lbs per acre	
Daphnia	—	Aquaria Immersion	—	- at 45^{0}F these invertebrates so insensitive that a TL_m could not be determined	63 (1957)
Daphnia	1.5 ppm	Aquaria Immersion	—	- TL_m at 50^{0}F 75 x greater than that of bluntnose minnow	
Gammarus	0.06 ppm	Aquaria Immersion	—	- TL_m at 55^{0}F	
Ephemera (Mayfly)	10 ppm	Aquaria Immersion	—	- TL_m (24 hour)	
Invertebrates (Various)	0.1 ppm	Lake Treatment	One month	- a large proportion killed by this exposure, but re-population occurred within a year	
Various Invertebrates	0.001 - 0.05 ppm	Aquaria	—	- Enchytraeen larvae, Corethra larvae, Gammarus, Asellus and Daphnia were generally resistant at concentrations tested - fish exposed to sublethal dose were usually killed by feeding on toxaphene-containing food animals	108 (1960)

These findings indicated, as did similar data from rat studies, that there was a significant difference in the toxicity of various components of commercial toxaphene. Turner et al.(16) also found differences in the toxicity of toxicant A and B and postulated that they contributed significantly to the overall toxicity of the insecticide to the house fly.

Toxaphene has been shown to be effective in controlling many insects, including the house fly. Published topical LD_{50} values for the house fly ranged from 31 (9)(Table 7-3); (98)(Table 7-4) to 70 mg/kg (5)(Table 7-5); (19)(Table 7-6) which probably results from differences in test methodology or the age and weight of insects used.

Technical toxaphene was separated into 8 fractions by column adsorption chromatography on alumina, using a graded solvent elution sequence and one component had the ring structure of camphene (9). The toxicities of the 3rd and 7th alumina column fractions as well as compounds I and II were determined by Landrum et al.,(9)(Table 3-7) using the mouse and housefly as the test organisms. Column fraction 3 had approximately the same toxicity in both the mouse and housefly as crude toxaphene, whereas fraction 7 was 1.5-2 times less toxic to both test systems than toxaphene itself. Components 1 and 3 were found to have the same order of magnitude of toxicity as crude toxaphene in the housefly system. The investigators pointed out that there were at least two basic types of ring structures in toxaphene, polychlorobornanes and polychlorodihydrocamphenes. They noted that other major components, not necessarily of these basic structures, could also be present and that they could have different selective toxicities.

As was discussed in detail previously, others have also noted the difference in toxicity of various fractions of toxaphene and it has been pointed out that the differences were not reflected in the extent of their metabolism, which appeared similar for all fractions (19)(Table 7-6).

Table 8: Summary Of Fish Acute Toxicity Studies

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
1.	Various fish	0.25 mg/1 (ppm)	Immersion	—	- effective eradicating fish in warm water systems	129 (1949)
2.	Various fish	—	Immersion	—	- as a piscicide, effective application varies with conditions and fish species involved (usually ranged from 0.02 - 0.1 mg/1)	80 (1971)
3.	Bluegill, Large-mouth Bass Fingerlings	0.05 ppm	Aquaria Immersion	36 hours	- in aquaria all fish were killed within the stated times	55 (1950)
	Goldfish	0.05 ppm	Aquaria Immersion	156 hours	- in aquaria all fish were killed within the stated times	
	Bluegill, Large-mouth Bass Fingerlings	0.05 ppm	Earthen Pond	5 weeks	- when identically treated but in an earthen pond, no mortalities were recorded within a 5-week period	
	Bait-size Goldfish	0.2 ppm	Earthen Pond	45 hours	- all fish died within 45 hours - this pond remained toxic for at least 10 weeks	
4.	Spot, Leiostonus xanthurus	3.2 ppb	Aquaria Immersion	24 hours	- all fish killed	126 (1964)
		0.1 & 0.01 ppb	Aquaria Immersion	5 months	- no effect detectable. These fish were then exposed to higher doses and found to be slightly more sensitive than fish receiving identical exposure which had never seen toxaphene before	
		0.5 - 3 ppb	Aquaria Immersion	48 hours	- at 0.5 ppb no fish died within 48 hours - at 1 ppb and above, 60 - 100% of fish died within 48 hours	
5.	Goldfish	0.025 ppm	Aquaria Immersion	24 hours	- TL_m (24 hour)	111 (1953)
		<0.005 ppm	Aquaria Immersion	10 days	- TL_m (10 day)	

Table 8: continued

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
6.	Carp, Bonytail Lg. Mouth Bass, Bluegill, Brown Trout, Bullhead Catfish	0.1 ppm	Immersion in Natural Lake	48 hours	- carp died within 12 hours - fish kill continued for 48 hours - one year later experimental stocking of lake with trout was successful (shallow lake, 65°F)	64 (1954)
7.	Various fish species	0.005 - 0.2 ppm	Immersion	—	- lower limits of toxicity depending on fish species, water temperature, hardness, location and duration of test - effect of pH unclear - toxaphene is more toxic in hard water	63 (1957)
	Goldfish, Large Mouth Bass, Bluegill	0.05 ppm	Immersion in Aquaria or Pond	—	- all fish died in aquaria but not when tested in an earthen pond	
	Goldfish, 10 various spp.	0.02 ppm	Immersion	—	- goldfish not killed, but other species tested were killed	
	Trout Fingerlings	0.005 ppm	Immersion	—	- all fish killed within 3 hours	
	Carp	0.05 - 0.2 ppm	Immersion	—	- all fish killed (field trial)	
	Bluntnose minnow (Pimephales spp.)	0.02 ppm	Immersion	—	- 55°F TL_m	
	Various fish species	0.05 ppm	Lab trials or lake treatment	—	- sufficient for fish eradication (emulsified toxapnene) - lower concentrations can be used successfully in shallow, hard-water lakes having a high temperature range (70 - 80°F)	

Table 8: continued

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
8.	Fathead Minnows	7.5 ppb	Aquaria Immersion	—	) - 96-hour TL_m at 25°C in soft water at pH 8.0 - toxaphene	93 (1959)
	Bluegills	3.5 ppb	Aquaria Immersion	—	) somewhat more toxic in hard water - more toxic to fish	
	Goldfish	5.6 ppb	Aquaria Immersion	—	) tested than DDT - Endrin was the most toxic chlorinated	
	Gupplies	20 ppb	Aquaria Immersion	—	) hydrocarbon tested	
	Fathead Minnows	5.1 ppb	Aquaria Immersion	—	- 96-hour TL_m at 25°C in hard water at pH 8.0	
9.	6 Freshwater spp.	0.01-0.05 ppm	Aquaria Immersion & "field trials"	—	- 6 freshwater species tested (Cyprinus sp., Tinca sp., Carassius sp., Leuciscus sp., Perca sp., Gobio sp.) were extremely sensitive - carp were the most resistant of this group	108 (1960)
10.	Channel Catfish	13 ppb	Aquaria Immersion	96 hours	-	109 (1970)
	Black Bullhead	5 ppb	Aquaria Immersion	96 hours	-	
	Goldfish	14 ppb	Aquaria Immersion	96 hours	- 96-hour TL_m	
	Carp	4 ppb	Aquaria Immersion	96 hours	-	
	Bluegill	18 ppb	Aquaria Immersion	96 hours	- Considerable variation noted in sensitivity to toxaphene	
	Bass	2 ppb	Aquaria Immersion	96 hours	- among members of five different families	
	Rainbow Trout	11 ppb	Aquaria Immersion	96 hours	-	
	Brown Trout	3 ppb	Aquaria Immersion	96 hours	-	
	Coho	8 ppb	Aquaria Immersion	96 hours	-	
	Perch	12 ppb	Aquaria Immersion	96 hours	-	
11.	Bullhead, Carp	0.1-0.42 mg/l	Lake Treatment	—	- bullhead and carp populations were eradicated	43 (1973)
		0.04-0.15 mg/l	Lake Treatment	—	- remained toxic to fish from a few months to 5 years after treatment - persistence associated with deep, soft water, biologically sparse lakes intended for trout stocking	
12.	Mosquito fish	0.008 ppm	Immersion	96 hours	- TL_m (96 hour) toxaphene	110 (1975)

Table 8: continued

SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
Grass Shrimp	860 ppb	Aquaria	96 hours	- TL_m (96 hour) toxaphene	110 (1975)
Crayfish	0.21 ppm	Aquaria	95 hours	- TL_m (96 hour) toxaphene	
Mactrid Clam	46.0 ppm	Aquaria	96 hours	- TL_m (96 hour) toxaphene	
13. Mosquito Fish (Gambusia affinis)	860 ppb	Immersion	24 hours	- LC_{50} (24 hour) toxaphene	91 (1976)
	2 ppm	Immersion	12 hours	- LT_{50} toxaphene	
	0.6 ug/g	Immersion	8 hours	- caused death in most of population	
	0.2 ug/g	Immersion	8 hours	- no effect level (apparently health fish)	
14. Trout	40 or 88 ug/l	Lake Treatment	—	- in deep lake, fish (trout) could not be restocked for 6 years, whereas in a shallow lake restocking after one year was successful - stocked fish concentrated toxaphene to levels approaching 14 mg/kg	30 (1966)
15. Juvenile Striped Bass	—	Aquarium with intermittent flow	4 days	- TL_{50} = 4.4 ug/l	130 (1974)
16. Black Bullheads, Rainbow Trout, Green Sunfish, Sm. Mouth Bass	0.05 ppm	Immersion (Lake)	—	- significant fish kill - "cleared lake" - symptoms of toxaphene poisoning noted when measured 8 to 15 ppm in the body tissue (0.028 ppm assayed following last addition of toxaphene) - after 31 days the lake concentration toxaphene = <0.001 ppm - lake was still toxic 9 months later, but not at one year	67 (1962)
17. Bluegill	3.5 ppb	Immersion	96 hours	- 50% kill in 96 hours (TL_m)	98 (1967)
Minnows	5.1 ppb	Immersion	96 hours	- 50% kill in 96 hours (TL_m)	
Goldfish	56 ppb	Immersion	96 hours	- 50% kill in 96 hours (TL_m)	
Guppies	20 ppb	Immersion	96 hours	- 50% kill in 96 hours (TL_m)	
Chinook Salmon	2.5 ppb	Immersion	96 hours	- 50% kill in 96 hours (TL_m)	
Coho Salmon	9.4 ppb	Immersion	96 hours	- 50% kill in 96 hours (TL_m)	
Rainbow Trout	8.4 ppb	Immersion	96 hours	- 50% kill in 96 hours (TL_m)	

In summary, using the topical house fly test, toxaphene had an LD_{50} of 70 mg/kg, whereas 7 fractions isolated by columns chromatography varied in toxicity from 42 to 316 mg/kg (19)(Table 7-6).

Specific examples were published highlighting these differences in toxicity. For toxaphene, toxicant A (a $C_{10}H_{10}Cl_8$ compound) and toxicant B (a $C_{10}H_{11}C_7$ compound) the LD_{50}'s using the topical house fly test were 70, 16, and 32 mg/kg, respectively (15) (Table 7-8). Attention was drawn to the fact that other potent insecticidal components must have been present in technical toxaphene, since the toxicity of toxicants A and B did not account for the toxicity of the commercial formulation to the house fly.

Others have reported the toxaphene topical LD_{50} for the house fly to be 31 ug/g (93) (Table 7-9).

Atallah and Newsom (103)(Table 7-10) found that as low as 10 ug of toxaphene fed to adult lady beetles could cause significant mortalities in either diapausing or active insects. The LD_{50} was found to be 21.8 ug per active beetle (1789 mg/kg) and 44.2 ug per diapausing beetle (3619 mg/kg). It was also found that toxaphene interfered with the reproductive process, in that egg laying was inhibited.

The estimated LC_{50} for toxaphene to naiads of three species of stone flies was, on average, 18 ug/l at 24 hours, 7 ug/l at 48 hours and 2.3 at 96 hours (107)(Table 7-11).

McWhorter and colleagues (128)(Table 7-12) applied toxaphene at concentrations of 1 or 2 pounds per acre, to separate plots of corn to determine the persistence of the insecticide in controlling the European corn borer. The pesticide was effective through out the 8 day evaluation period and, in general, it seemed that granular insecticides were more effective than spray products.

Using various chromatographic methods Nelson and Matsumara (18) (Table 7-13) isolated toxic fraction A from toxaphene. Toxin A was subsequently found to be 1.5-2 times as toxic as toxaphene itself towards mosquito larvae, shrimp and algae. The toxaphene components were partially characterized to be octachlorobornanes. The authors were of the opinion that information on the toxicity of the various toxaphene components permitted the assessment of the fate of the individual components, as well as the environmental implication of the use of toxaphene.

Adkisson (105)(Table 7-14) estimated that the amount of toxaphene required to control the tobacco budworm had increased 6-fold in recent years due to the development of resistance by the insect. This has lead to increased production costs and further aggravation of the ever increasing problem of environmental contamination by pesticides.

6.2.4 Fish

Since the accidental discovery that toxaphene was effective in eradicating fish populations, it has been used for controlling "rough" fish under a variety of conditions. A summary of toxicity of toxaphene to various freshwater fish can be found in Tables 8 and 9.

Surber (129)(Table 8-1), in 1949, found that 0.25 mg/l (ppm) was effective in eradicating fish in warm water systems.

In a discussion on the use of toxaphene as a piscicide it was pointed out that the effective application rate varies according to conditions and to the fish species involved but usually ranged from 0.02 to 0.1 mg/l (80)(Table 8-2). An undesirable feature of toxaphene is its persistence and slow rate of detoxification in some lakes. Lakes treated with toxaphene usually required 2 to 12 months before detoxification reduced the concentration of the chemical to a level suitable for restocking. However, instances of lakes remaining toxic for periods up to 4 years have been recorded.

In 1950, Lawrence (55)(Table 8-3) reported on the toxicity of toxaphene to fish. At that time toxaphene had only been in use for 4 years but its effectiveness in the control of the boll weevil was well known and the use of the insecticide was increasing rapidly in the cotton-belt states. Bluegill and large-mouth bass fingerlings, stocked in aquaria, were killed in 36 hours by 0.05 mg/l (ppm) of toxaphene. At this same concentration, goldfish died in 156 hours; during the last 105 hours the goldfish were inactive, lying on their sides and barely moving their gill covers or fins. However, in a small earthen pond, 0.05 mg/l of toxaphene did not kill blue-gill fingerlings, bass fingerlings or bait-sized goldfish within a 5-week period. Lawrence noted that each acre of cotton received 10 pounds of 20% toxaphene dust per application and, because it was usually applied by airplane or power dusters, there was a considerable drift of dust onto areas adjoining the treated locations. He estimated that if one regular field application of toxaphene were applied to an average one acre pond, it would result in approximately 0.2 mg/l of toxaphene in the pond water. This concentration was added to a small earthen pond containing blue-gills, bass fingerlings and bait-sized goldfish, and all fish died within 45 hours. Five weeks later the pond was restocked with bluegill fingerlings. The majority of the fish survived for three weeks, but all were dead by the end of six weeks. The water remained toxic for 10 weeks but no indication was given as to when the pond was sufficiently clear of toxaphene to permit survival of the fish.

The acute toxicity of toxaphene in a constant flow system using sea water and Leiostonus xanthurus was investigated by Lowe (126) (Table 8-4). At concentrations of toxaphene above 3.2 ug/l (ppb), all fish were killed within 24 hours. Eight out of 10 fish died within 144 hours at a concentration of 0.5 ug/l.

Soil, which had been treated with toxaphene in August, sampled in September and analyzed in November-December, was tested for its toxicity to fish (111)(Table 8-5). In these experiments, goldfish were held in 10 litres of water to which 1 kg of toxaphene contaminated soil had been added. In one instance, the soil from a field had reportedly received 84 pounds of a 20% toxaphene dust per acre and no deaths were recorded. Another sample, from a field treated with about 108 pounds per acre, resulted in 2 of 4 goldfish dying within a week. A third sample, from a field said to have been treated with only 30-31 pounds of toxaphene dust per acre in 3 dustings proved most toxic, killing 7 of 8 goldfish within 7 days. An aqueous extract of the same soil, prepared by filtering the suspension, proved at least as toxic as the unfiltered mixture of water and soil with a 100% mortality occurring within 6 days in four goldfish. In a natural surface pond, which had an average depth of 9 feet, the first application of toxaphene at a rate of 0.2 pounds, or about 0.008 mg/l, apparently killed no fish (111); but two applications (0.016 mg/l toxaphene) killed all the fish. In another pond, averaging 3-4 feet deep, the first application at a rate sufficient to give a final concentration of about 0.01 mg/l killed most of the fish present, and three such treatments resulted in a 100% mortality.

Goldfish survived for 10 days in a concentration of 0.0032 mg/l of toxaphene, but 7 of 9 were killed in 10 days by 0.0056 mg/l and all died in water containing 0.01 mg/l (111). A 10-day median tolerance limit was calculated to be somewhat below 0.005 mg/l. The concentration of toxaphene at which 50% of the goldfish showed persistent loss of equilibrium after a 24-hour exposure was estimated to be about 0.025 mg/l.

Hemphill (64)(Table 8-6) investigated the effect of toxaphene as a fish toxin by using a reservoir treated with the chemical to achieve a concentration of approximately 0.1 mg/l. Carp became distressed and began dying within 12 hours. The die-off continued for 48 hours with a kill of 220 tons of carp, 3,000 pounds of bonytail, 29 large-mouth black bass, 31 bluegill, 17 brown trout and several hundred bull-head catfish. In a second lake a similar concentration of toxaphene was applied and within 11 days no fish activity was evident. Over the following two years the lake was periodically sampled and no "rough" fish were observed. One year later, an experimental plant of rainbow trout proved to be successful, and the fish attained normal size in the usual growing period.

The use of toxaphene as a fish poison was also reported by Hooper and Grzenda (63)(Table 8-7). The 24-hour TL_m for toxaphene ranged from 0.0057 mg/l in the blunt-nosed minnow to 9.5 mg/l in Ephemera cimulans (Mayfly nymphs). The toxicity to minnows increased significantly between water temperatures of 50°F and 75°F and toxaphene was more toxic to fish in hard water than in soft water. In addition, many groups of invertebrae were killed by the toxaphene treatment, but they reappeared even though the lake water remained toxic to fish. The data indicated that toxaphene was more toxic to fish than to many aquatic invertebrates. The investigators suggested that since the bottom invertebrates reappeared in the lake before it lost its toxicity to fish, food organisms were likely to be present by the time the lake was ready for restocking.

The relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish were examined. Bioassays on fish were conducted by Henderson et al. (93)(Table 8-8) at a temperature of 25°C, and the 96-hour TL_m (median tolerance limit), determined. Endrin was the most toxic of the insecticides studied to all species of fish which included fathead minnows, bluegills, goldfish and guppies. Toxaphene was the second most toxic insecticide and the 96-hour TL_m ranged from 3.5 ug/l (ppb) to 20 ug/l in bluegills and guppies, respectively. The value for the fathead minnow was 5.1 ug/l. Comparative testing in soft and hard water suggested that toxaphene was somewhat more toxic in hard water.

The investigator commented that fish bioassays cannot be used to predict toxicity to mammals and that, conversely, mammalian data cannot be used to predict toxicity in fish because the values obtained were not camparable due to the different modes of administration.

Koppe (108)(Table 8-9) carried out numerous aquarium experiments using fresh water fish, their food animals and larvae to examine the poisonous effects of toxaphene. Fish proved to be extremely sensitive to the smallest amount of toxaphene used, (0.001 to 0.05 mg/l) with carp and krucian carp being most resistant, compared to other fish. The temperature affected the course of the poisoning, but not the lethal dose. Fish could recover in toxaphene-free water after a short exposure to lethal doses. Food animals were generally resistant to toxaphene, particularly enchytraeen and corethra larvae. Feeding experiments with poisoned food animals showed that the fish stopped feeding when small damages occurred and recovered thereafter. Fish exposed to sublethal doses or weakened by parasites were usually killed by feeding on the poisoned food animals. In general, two field experiments conducted during different seasons confirmed the results of the aquarium experiments.

The 96-hour TL_{50} for toxaphene ranged between 1.5 ug/l for brown salmon, 1 ug/l for bass and up to 14 ug/l for goldfish and minnows, in experiments designed by Macek and McAllister (109)(Table 8-10).

Although it is thought that water hardness influences toxaphene toxicity and its rate of detoxification, the mechanisms of the effect of the minerals content of water is not well understood. Field trials and laboratory experiments indicate that a concentration of 0.05 mg/l of emulsified toxaphene is sufficient for fish eradication, but low concentrations are effective in shallow, hard water lakes having a high temperature range of 70° to 80°F (63)(Table 8-7).

Bullhead and carp populations were eradicated from four different lakes with concentrations of toxaphene ranging from 0.1 to 0.42 mg/l (43)(Table 8-11). Some lakes treated with the piscicide at the usual concentrations used for fish eradication (0.4 - 0.15) mg/l), remained toxic to fish for periods of a few months to 5 years after treatment. The persistence of toxaphene was associated with deep, soft-water, biologically sparse lakes intended for trout stocking (43).

During a study of the toxicity of toxaphene and herbicides on four species of aquatic organisms including mosquito fish, grass shrimp, crayfish and the mac trid clam, the 24, 48, 72 and 96 hour medium tolerance limits (TL_m) were determined for each. The 96-hour TL_m for mosquito fish was 0.008 mg/l; for grass shrimp, 0.036; for cray fish 0.21 and for clam, 460 mg/l. Except for the clam, toxaphene was the most toxic compound studied which included carbaryl, propanil and molinate (110)(Table 8-12).

Schaper and Crowder (91)(Table 8-13) determined an LC_{50} at 24 hours of 860 ug/l (ppb) for mosquito fish. At 2 mg/l (ppm) the LT_{50} was 12 hours, but 8 hours was selected as the optimum period for uptake and excretion experiments, since mortality increased rapidly after 8 hours at this concentration. The fish were placed in aquaria containing 2 mg/l of toxaphene and ^{36}Cl-toxaphene. The fish were removed at various times after exposure and the radioactivity in the fish measured. The amount of toxaphene itself was not analyzed, although values were presented as specific activities. The investigators divided the toxicity symptoms due to toxaphene into five arbitrary stages. The first stage was recorded as apparently healthy fish with the second stage being when the fish began to swim perpendicular to the surface. It was difficult to determine whether this was a toxicity syndrome, because swimming at the surface was normal in water with low oxygen and it was also the normal feeding position for the fish. Subsequent toxicity stages were all marked by rapid gill ventilation, which further suggested respiratory involvement. The third stage was characterized by fish swimming parallel to the side of the aquaria, but with a loss of equilibrium as evidenced by sinking of the posterior end. Finally, fish lost their ability to maintain their normal dorso-ventral orientation and rolled to the side. At stage four, fish sank to the bottom and were prostrate with rapidly ventillating gills. At this stage there was occasional darting behaviour. Death, or stage five, was identified by the cessation of gill movements. On average, stage 1 occurred at concentrations of 0.2 ug/g of fish, stage 2 at

0.5, stage 3 at 0.6 ug/g, with similar values being found for stages 4 and 5. The onset of toxicity symptoms varied within any given population, and the onset of particular symptoms were directly proportional to the body load. Thus within this population, differences in toxicity response appeared to be due to body loads, rather than to different tolerances to a particular toxicant level.

The toxicity of two lakes treated with toxaphene at estimated concentrations of 40 or 88 ug/l were investigated by Terriere et al.,(30)(Table 8-14). In a deep lake the fish could not be restocked for six years, whereas in a shallow lake fish were reintroduced within 1 year and the trout grew rapidly. The levels of toxaphene in both lakes decreased rapidly to less than 1 ug/l within a few months, and then remained at that level for a few years. The fish stocked into the lake concentrated the toxaphene to levels approaching 14 mg/kg. Although the analytical methodology used in this study was not particularly sensitive, it did indicate that fish placed in low levels of toxaphene-containing water concentrated the pesticide.

The TL_{50} of toxaphene to juvenile striped bass was reported as being 4.4 ug/l (130)(Table 8-15). This value was intermediate between the toxicity of endrin, heptachlor and DDT, which were lower;and those of lindane, chlordane and methylparathion, all of which had higher TL_{50}'s. These tests were carried out using a 4-day assay period with proportional dilutors and intermittent flow conditions, which the authors considered as being more meaningful than static tests.

The toxicity of toxaphene to fish (Table 9) probably varies because fish react differently in waters of different chemical composition and temperature. By any estimation, however, toxaphene must be classified as an extremely potent poison for fish. Symptoms of toxaphene poisoning of fish are fast, erratic swimming, occasional bumping into objects,with a general increase in locomotory efforts accompanied by a deterioration in coordination. This spasmotic

Table 9 TOXICITY TO VARIOUS FRESHWATER FISHES (STATIC BIOASSAYS) (From Ref. 102)

Fish Species		96-hour LC50 (ug/l)
Largemouth bass		2
Brown trout		3
Bluegill		3.5
Carp		4
Black bullhead		5
Goldfish		5.6
Coho salmon		8
Rainbow trout		11
Yellow perch		12
Channel catfish		13
Redear sunfish		13
Goldfish		14
Fathead minnow (hard water)		5.1
Fathead minnow (soft water)		7.5
Guppies (soft water)		20
Chinook salmon		2.5
Coho salmon		9.4
Rainbow trout		8.4
Rainbow trout	(53°F)	8.4
Stonerollers	(53°F)	14
Stonerollers	(73°F)	<5
Goldfish	(53°F)	94
Goldfish	(73°F)	50
Golden shiner	(73°F)	6
Bluntnose minnow	(53°F)	30
Bluntnose minnow	(73°F)	6.3
Black bullhead	(53°F)	25
Black bullhead	(73°F)	1.8

activity gradually diminishes and an apparent paralysis sets in, indicating disruption of the somatic nervous system. Depending on water temperature, the fish then settle to the bottom or float on their sides with their backs arched laterally (43).

6.2.5 Plankton And Algae

Hoffman and Olive (114)(Table 10-1) studied the effect of 0.1 mg/l of toxaphene on the plankton in water reservoirs. Their data suggested that the fish toxicant accelerated the decline in numbers of protozoans in the treated lakes. Since the protozoans did not immediately repopulate the treated reservoirs, as was the case in a control reservoir, it was concluded that toxaphene was inhibitory to protozoan growth. In addition, toxaphene reduced the size of the rotifer population in the treated reservoirs.

The effect of several insecticides on marine phytoplankton, which were considered important foods for oysters and clams, was investigated by Ukeles (113)(table 10-2). Toxaphene inhibited the growth of one organism at 0.01 mg/l and three out of four others at 0.07 mg/l. The growth of all five organisms studied was inhibited at 0.15 mg/l.

The effect of toxaphene on the growth and ^{14}C assimilation from sodium carbonate in fresh water algae (Scenedesmus) was reported by Stadnyk and co-workers (112)(Table 10-3). Initial concentrations of 0.1 and 1.0 mg/l of toxaphene were used. It was suggested that these levels would decrease rapidly with time through pro-distillation and adsorption to surfaces of the containers. (No measurements of the toxaphene concentration in the growth medium were made). A concentration of 0.1 mg/l of toxaphene caused a small fall (13%) in cell number after 4 days, and 1 mg/l resulted in a decrease in cell number between 15 and 25% after two to ten days treatment. There was a 300-400% increase in carbon fixation with 1 mg/l of toxaphene and a 25-50% increase at the 0.1 mg/l level. This contrasted with DDT which caused a fall in carbon assimilation. The authors commented that their study emphasized the need to consider long-term chronic effects of pesticides in the ecosystem in terms of plant life as well as animal species. They also noted that small additions of persistent organochlorine insecticides may cause a reduction in population density and a supression of carbon fixation although it was unlikely that toxaphene would have a serious effect on algae in these respects.

Nelson and co-workers (17, 18) examined the chemical nature and acute toxicity of the toxaphene toxic fraction A in mosquitoe larvae, brine shrimp and algae. They found that toxic fraction A was 1.87, 1.75 and 1.35 times more toxic than toxaphene itself to mosquitoe larvae, brine shrimp and algae respectively. It was notable that Nelson and Matsumura (18) found several other toxaphene fractions which were particularly toxic for the brine shrimp and algae.

6.2.6 Plants

Investigations into the effect of toxaphene on tree foliage carried out in 1948 showed that 0.4% aqueous suspensions were harmless to some 70 species of trees and shrubs and the material appeared to be non-phytotoxic in most combinations, except to cucurbits, which sometimes had extensive damage. Although there have been some indications that low concentrations of toxaphene may have depressed seedling growth, it did not injure crops when present in soil at 25 pounds per acre (2)(Table 10-4).

However, Cullinan (115)(Table 10-5), in a paper in which no experimental details were given, commented that toxaphene depressed the growth of some seedling plants when applied to the soil, even at low levels (25 pounds/acre). Plant growth was inhibited even more with a higher soil concentration and the author mentioned that root development may be retarded with some plants. The toxicity of toxaphene appeared to decrease with time, which was interpreted as being due to destruction by soil fungi or bacteria.

Toxaphene, in concentrations of 0.3, 0.15 and 0.07%, inhibited photosynthesis in alfalfa (116)(Table 10-6). Kralovic reported that assimilation processes of alfalfa were more than 1/3 lower with toxaphene treatment than in control material, and the inhibiting effect of toxaphene on photosynthesis could be limited by precipitation. The effect of artificial rain reduced the toxaphene effect, even when carried out one day after insecticide application. It was noted that toxaphene increased seed production in pods, although it had a negative effect on photosynthesis in alfalfa.

In some cases, co-formulation of toxaphene with other materials has caused varying degrees of phytotoxicity. Thus, admixtures with Bordeau mixture, calcium arsenate, ferrous sulphate, lime, lime-sulphur, or summer oils was not recommended. Mixtures with oil particularly have caused damage to fruit tree foliage. (2)(Table 10-4).

6.3 Sub-Acute Toxicity

Very few studies into the sub-acute toxicity of toxaphene appear to have been reported in the literature. Relevant investigations which were reviewed are summarized below.

6.3.1 Mammals

The cumulative LD_{50} for male rats was found to be 700 - 2,000 mg/kg when administered intraperitoneally every 48 hours over a 10-12 day period (19)(Table 11-1).

Cows which were restricted on 4 occasions for 60 days each to drinking lake water, which had been treated with 0.1 mg/l (ppm) of toxaphene, exhibited no toxic symptoms. It was estimated that a cow would have to drink 18,000 gallons of water a day from such a lake in order to ingest a toxic dose of toxaphene (64)(Table 11-2).

In another study using dairy cattle, Claborn et al. (87)(Table 11-3) fed the animals toxaphene in their diet for 8 weeks at levels of 20, 60, 100, and 140 mg/kg (ppm). The pesticide was excreted

Table 10: Summary Of Plankton, Algae And Plant Acute Toxicity Studies

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
1.	Freshwater Protozoans	0.1 ppm	Immersion	—	- accelerated a decline in protozoan numbers and reduced size of rotifer population	114 (1971)
2.	Marine Phytoplankton (5 species tested)	0.1 - 0.15 ppm	Immersion	—	- 0.01 ppm tolerated by four species - 0.15 ppm was lethal to all organisms - 0.001 ppm proved lethal to *Monoschripis lutheri*	113 (1962)
3.	Freshwater algae (*Scenedesmus*)	0.1 - 1.0 mg/l	Immersion	10 days	- 1.0 mg/ml caused nearly a 25% decrease in cell nos. after 10 days treatment - no overall significant effect at 0.1 ml/l (No *in situ* measurements of toxaphene were made)	112 (1961)
4.	70 species of trees and shrubs	0.4% aqueous suspension	Foliage Spray	—	- generally non-phytotoxic - extensive damage caused to curcubits	2 (1974)
5.	Various species seedling plants	25 lbs/acre	Applied to soil	—	- depressed growth - root development retarded with some species at higher toxaphene conc.	115 (1949)
6.	Alfalfa	0.3, 0.15, 0.07%	Foliage Spray	—	- initial reduction photosynthesis to 1/3 normal - inhibition reversed by rainfall - toxaphene penetrated leaves	116 (1975)
7.	Protozoa and Phytoplankton	0.01 - 0.001 ppm	Immersion	—	- these concentrations were generally toxic	12 (1973)

in the milk, but no clinical signs of poisoning were evident.

Two dogs were administered 10 mg/kg (ppm) of toxaphene in a capsule on a daily basis. One dog died after 33 days but the second one lived for 3.5 years. Four dogs were given 5 mg/kg (ppm) and all survived for 4 years, at which time they were sacrificed. No information on the pathologic findings were reported. (131)(Table 11-4).

6.3.2 Birds

Genelly and Rudd (120)(Table 11-5) fed toxaphene to ring-necked pheasants in their diet at levels of 25, 100, 200, and 300 mg/kg (ppm) for 50-94 days. No mortality was observed at any of the doses used. Although some of the pheasants lost weight,this may have been due to a dislike of the contaminated food.

Heath et al. (cited in 76) reported the 5-day dietary LC_{50} to be 96-142 mg/kg for 4 species of young birds.

6.3.3 Fish

Fathead minnows,exposed to concentrations of toxaphene between 55 and 1230 ng/l (ppt) in a flow through system for 150 days, showed no changes in growth for up to 90 days; but within 150 days growth was significantly reduced at all concentrations (89)(Table 11-6). After 150,days toxaphene residues in the minnows were 5.9, 13, 22; and 52 ug/g (ppm) in fish exposed to 55, 132, 288, and 621 ng/l (ppt), respectively. Mehrle and Mayer pointed out that only fish from the southern U.S. ordinarily contained toxaphene residues and the residues most likely resulted from agricultural run-off. The minnows exposed to 55 and 132 ng/l (ppt) of toxaphene had residues in the range reported in fish by the U.S. National Pesticide Monitoring Programme (1974), which ranged from 0.5 to 48 ug/g (ppm) with an average value of 8.7. It was also found that the collagen content of the back bone of the minnows exposed to toxaphene was decreased, the amino acid composition changed and the calcium

Table 11: Summary Of Sub-Acute Toxicity Studies

SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
1. Rat	50 mg in methoxy triglycol	Intraperitoneal	48 hr intervals for 10 - 12 days	- LD_{50} for cumulative toxaphene dose was 700 - 2,000 mg/kg - considerable toxaphene was unmetabolized and remained as a lump near the site of injection	19 (1975)
2. Cow	0.1 ppm	Oral	Daily for 60 days	- no toxic symptoms noted (to be toxic, a cow would have to drink 18,000 gallons x 0.1 ppm toxaphene per day)	64 (1954)
3. Dairy Cow (Jersey)	20 - 140 ppm in diet	Oral	8 weeks	- toxaphene levels in milk ranged from 8 to 24.3 ppm - 1 week after toxaphene feeding ceased 0.1 - 0.3 ppm found in milk - there were no clinical signs of poisoning in any animals	87 (1963)
	0.5% spray	Topical	Twice at 3-week intervals	- concentrations in milk were maximum after first spray (0.61 ppm) [spray = emulsion (wettable powder or in oil)]	
	1 oz. of 2% oil spray	Topical	Daily for 21 days	- toxaphene levels in milk maximum after three days treatment (0.41 ppm) - no signs of clinical poisoning noted	
4. Dogs	10 mg/kg (ppm)	Oral	Daily for life	- one dog died after 33 days, but the other lived for 3.5 years	131 (1952)
	5 mg/kg	Oral	Daily for life	- all 4 dogs survived for 4 years, then were sacrificed (no information on pathological findings were reported)	
5. Ring-necked Pheasant	25, 100, 200, or 300 ppm in diet	Oral	51 - 94 days	- no mortalities recorded - some females at the highest dose level lost weight	120 (1956)
6. Fathead Minnow (*Pimphales promelas*)	55 & 1230 ng/l	Immersion	90 days	- growth not affected	89 (1975)
	55 & 1230 ng/l	Immersion	150 days	- growth significantly reduced	
7. Rat	25 mg	Intraperitoneal	Daily for 7 days	- looked for and found an increase in the rate of estrone metabolism	119 (1971)

Table 11: continued

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
8.	Ring-necked Pheasant	100 & 300 ppm in diet	Oral	Daily for 8 wks	- 100 ppm treated birds consumed 4.14 mg toxaphene per day - the 300 ppm birds consumed 9.12 mg/kg - egg production decreased in 300 ppm treated birds - egg production, fertility and hatchability not affected significantly in 100 ppm treated birds	122 (1956)

content increased. It was suggested that toxaphene altered the development and quality of the back bone and induced biochemical manifestations of the "broken back" syndrome.

6.4 Chronic Toxicity

There have been a number of studies published describing the chronic toxicity of toxaphene. Several of the seemingly more pertinent studies were reported in a 1968 FAO/WHO report on pesticides in food (69) from unpublished data not available to the authors of the present document. They are considered of such importance as to warrant discussion in this section. In general, the reports on the chronic toxicity of toxaphene provide conflicting findings. For example, one investigation found that 50 mg/kg (ppm) in the diet (123)(Table 12-1) caused some liver damage, whereas another study found 800 mg/kg (ppm) (95)(Table 12-2) without effect. The reasons for these differences in response to the agent are not immediately apparent.

6.4.1 Mammals

6.4.1.1 Rodents

In 1957, when the lowest observed dietary level of toxaphene producing a tissue change was considered to be 100 mg/kg(ppm); Ortega et al. (123)(Table 12-1) investigated the pathological changes in the livers of rats fed the pesticide at low levels. Male Sherman rats were fed toxaphene at a dose of 50 (3 mg/kg/day for adult rats*) or 200 mg/kg in their diet. Animals were serially killed at 2, 4, 6, and 9 months thereafter and histopathological examination of the liver carried out. With sufficiently careful examination it was possible, on occasion, to detect liver cell

*In this document an average rat weight of 350 g and a dietary intake of 20g/day were used to convert mg/kg of toxaphene in the diet to mg/kg body weight/day.

abnormality in rats receiving the 50 mg/kg (ppm) diet after 6-9 months exposure. Thus, it was suggested that the lowest level which produced minimal tissue change should be reduced to 50 mg/kg (ppm). At the same time, it was pointed out that the levels found to be toxic were probably minimal and were a threshold value where only a few animals would be susceptible. Unfortunately, only 1 rat per time point was examined (6 animals of each sex were used at each dietary level) and therefore, the significance of the findings is questionable.

In other studies, feeding toxaphene at rates of 100 mg/kg (ppm) and 800 mg/kg (ppm) in the diet of white rats and guinea pigs for a period of 6 months, showed no significant effect on growth, blood cell count, urinalysis, reproduction, mortality or histological findings (95)(Table 12-2).

The 1968 WHO monograph on toxaphene (69)(Table 12-3) reported the results of rat feeding studies by Treon of Kettering Laboratories, the details of which were unavailable to the authors of the present document. Four groups of 40 rats (20 males and 20 females) were fed 10, 100, 1000, and 1500 mg/kg (ppm) of toxaphene in their diet. Effects were determined by gross observation: mortality, body-weight, blood tests, liver weight, liver to body-weight ratios, gross autopsy and histological examination of the tissues. After 7.5 to 10 months of feeding, some of the rats fed 1500 mg/kg (ppm), and a few receiving 1000 mg/kg (ppm)(57 mg/kg body-weight/day) showed occasional convulsions. The body-weight gain of the rats fed the highest feeding level (1500 mg/kg (ppm)) for the first 20 weeks was less than those of the controls, probably due to decreased food intake because of the unpalatability of the food. As the animals became accustomed to the diet, their growth rate became equivalent to that of the control rats. There were no significant effects on mortality or on the hematopoetic system. The liver weight and liver to body-weight ratios were significantly increased only in the 1000 and 1500 mg/kg (ppm) groups. Liver changes consisted of swelling and homogeneity of the cystoplasm, with a peripheral arrangement of the granules in the cytoplasm of the centrilobular hepatic cells. These changes occurred to a moderate degree in the 1500 mg/kg (ppm) group and to a slight degree in the 1000 mg/kg (ppm) group (69)(Table 12-3).

In lifetime feeding studies, Fitzhugh and Nelson (132)(Table 12-4) fed rats toxaphene containing diets at concentrations of 25, 100 and 400 mg/kg (ppm). Histopathologic examination of liver tissue showed centrilobular hepatic cell enlargement, increased oxyphilia, peripheral margination of basophilic granules and a tendency to hyalinization of the remainder of the cytoplasm in animals receiving the 100 and 400 mg/kg (ppm) diets. Minimal effects occurred at 25 mg/kg or approximately 1.4 mg/kg body-weight/day which were demonstrated by minimal hepatic cell enlargement with peripheral migration of granules.

Industrial Bio-Test Laboratories and Hercules Incorporated (124) (Table 12-5) published the results of a reproductive study in albino rats during which parental animals were fed toxaphene in their diet for 39-42 weeks at 25 and 100 mg/kg (ppm). Rats were examined for gross pathologic changes at sacrifice, organ weights were recorded and tissues subjected to histopathological examination. Rats receiving the higher dose showed liver changes consisting of slight cytoplasmic fatty vacuolization. Other parameters were normal. No effect of any kind resulted from the administration of the 25 mg/kg dose.

6.4.1.2 Dogs

It was found that the cumulative effect of a daily dose of 5 mg/kg (ppm) administered to dogs would, after a few days, regularly cause convulsions (92)(Table 12-6). Therefore, for a chronic toxicity study, Lackey (92) chose a daily dose of 4 mg/kg and reported that on occasion convulsions were observed following this dose. Feeding two dogs for as long as 106 days gave evidence of cumulative effects for single daily doses. Gross pathologic changes were not observed and the blood picture remained normal, but degenerative disease changes in the liver parenchyma and in the renal tubules were evident on histologic examination.

In the same Kettering Laboratory unpublished report cited earlier (69)(Table 12-3), three male and five female dogs also received toxaphene 6 days a week in their diet for 2 years, starting at 4 months of age. The pesticide was added to the diet at 10 and 50 mg/kg (ppm) of the total wet diet including their liquids. The dogs received the daily equivalent of 0.60-1.47 mg/kg and 3.12-6.56 mg/kg (equivalent to 40 and 200 mg/kg(ppm) on a dry diet basis). Gross behaviour, body-weight, mortality, peripheral circulating blood elements, gross pathology, organ to body-weight ratios and histopathology were recorded.

No effects on behaviour, body-weight, mortality or blood elements were seen, but there were increases in liver weights, liver to body-weight ratios and moderate liver degeneration at the 200 mg/kg level. At 40 mg/kg, one of 3 dogs was reported to have slight liver enlargement and slight granularity and vacuolization of the cytoplasm. Re-examination of the sections of these animals failed to confirm any difference from control animals. All other tissues were normal at both feeding levels.

In a later study, Industrial Bio-Test Laboratories (cited in 69) (Table 12-3) fed groups of 12 dogs (6 males and 6 females) toxaphene at dietary levels of 0, 5, 10, and 20 mg/kg (ppm). Two male and 2 female animals were autopsied after 6, 12, and 24 months. None of the feeding levels caused any change in organ weights, or gross or histopathological appearance of tissues. None of the clinical or organ function tests measured were abnormal at any time.

In a U.S.A.-E.P.A. toxaphene criteria document (76)(Table 12-7) reference was made to an unavailable 1965 paper by A.J. Lehman, to the effect that the dog was the most sensitive animal to the

Table 12: Summary of Chronic Toxicity Studies

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
1.	Rat (Sherman Strain)	50 - 200 ppm in diet	Oral	Daily for 2 - 9 months	- occasionally noted liver cell abnormality at 50 ppm - suggested that commonly used no effect level of 100 ppm should be reduced to 50 ppm (feed was contaminated with DDT less than 0.5 ppm)	123 (1957)
2.	Rat	100 & 800 ppm in diet	Oral	Daily for 6 months	- no changes in growth, blood cell count, urinalysis, reproduction mortality or histological profile from normal	95 (1975)
3.	Rats	10, 100, 1000 & 1500 mg/kg (ppm)	Oral	7.5 - 10 months	- rats receiving more than 1000 mg/kg showed occasional convulsions - liver weight increased in 1000 and 1500 mg/kg groups - no significant effects on mortality or hemopoetic system - cytological changes of liver noted in 1000 and 1500 mg/kg groups included peripheral arrangement of cytoplasmic granules in centrilobular cells	69 (1968)
4.	Rats	25, 100 & 400 mg/kg (ppm)	Oral	Lifetime	- animals receiving 100 & 400 mg/kg diets showed centrilobular hepatic cell enlargement, increased oxyphilia, peripheral margination of basophilic granules and tendency to hyalinization of remainder of the cytoplasm - minimal hepatic cell enlargement, peripheral migration of granules in animals receiving 25 mg/kg (1.4 mg/kg body weight per day)	132 (1951)
5.	Rats (Sprague-Dawley)	25 & 100 ppm in diet	Oral	Chronic-3 generations	- at other concentration no effect on litter size, pup survival or weanling body weight - at 100 ppm parent animals showed fatty vacuolization in liver tissue - the no-effect level was judged to be 25 ppm - no teratogenic effects noted	124 (1973)
6.	Dogs	4 mg/kg	Oral	Daily for 106 days	- occasional convulsions observed following this dose - cumulative effects for daily doses - gross pathologic changes not observed and blood picture remained normal - degenerative disease changes in liver parenchyma and renal tubules were evident on histologic examination	92 (1949)

Table 12: continued

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
7.	Dog	—	Oral	—	- most sensitive animal to chronic administration of toxaphene - highest level of minimal or no long-term effect was 10 mg/kg in diet or 1.7 mg/kg body weight per day	76 (1976)
8.	Rainbow Trout (Salmo gairderi)	0.4 - 0.66 ppb	Immersion	280 days	- survived with no effect on growth	75 (1967)
		1 ppb	Immersion	14 days	- LC_{100} toxaphene	
	Atlantic Salmon (Salmo salar)	0.4 - 0.66 ppb	Immersion	225 days	- survived with no effect on growth	
		1 ppb	Immersion	14 days	- LC_{100} toxaphene	
9.	Fish	0.1 & 0.01 ug/l (ppb)	Immersion	5 months	- no significant difference in mortality between test and control groups at both concentrations - chronically exposed fish were more sensitive than control to subsequent acute exposure to 1 ug/l (ppb)	126 (1964)
10.	Mouse	25 ppm in diet	Oral	5 - 6 chronic generations	- little or no effect noted	125 (1968)
11.	White Leghorn Hens	10 or 100 ppm in diet	Oral	24 weeks	- no significant effect on egg laying, fertility, hatchability, survival of progeny	121 (1976)

chronic administration of toxaphene. The highest level found to have minimal or no long-term effects was supposedly 10 mg/kg in the diet or 1.7 mg/kg of body-weight per day.

6.4.1.3 Monkeys

The Kettering group (69)(Table 12-3) fed two adult, female monkeys toxaphene in their food 6 days a week for 2 years at a dose of 0.64-0.78 mg/kg/day. A third animal served as a control. There was no evidence of intoxication or tissue or organ damage, as measured by growth rate, liver and spleen to body-weight ratios and by histological examination of the tissues (69)(Table 12-3).

6.4.2 Fish

Newsom (75)(Table 12-8) in a review article, referred to studies in which rainbow trout were exposed to 0.4-0.66 ug/l (ppb) and which survived for at least 280 days. No effect on growth was noted and the fish had a toxaphene content of 13.7 mg/kg (ppm). The LC_{100} was published as being 1 ug/l (ppb) for 14 days exposure. A similar LC_{100} was reported for Atlantic salmon, which survived 255 days at 0.4-0.66 ug/l (ppb) with no effect on their growth. Body concentrations of toxaphene were reported as 5.5 mg/kg (ppm).

Groups of fish were exposed to 0.1 and 0.01 ug/l (ppb) of toxaphene for a 5 month period. These concentrations proved to be below the lethal level and the number of experimental fish dying did not differ significantly from the controls. At the end of the chronic study, fish were exposed to acute concentrations of toxaphene of either 0.5, 1, 2, or 3 ug/l (ppb) and it was found that the fish, which had been previously exposed to toxaphene, were as sensitive or more sensitive to these lethal concentrations of toxaphene than the control fish. No fish died within 48 hours when exposed to 0.5 ug/l (ppb). At 1 ug/l (ppb) and above, 60-100% of the fish were dead within 48 hours (126)(Table 12-9).

6.5 Dermal Toxicity

The effect of the topical application of toxaphene to dogs and rabbits was studied by Lackey (118)(Table 13-1). The application of a daily dose of 200 mg/kg for 22 days to 600 mg/kg for 30 days did not have any adverse effect on dogs. At autopsy, however, the livers showed degenerative changes similar to those seen when toxaphene was fed to the animals orally. In the rabbit, however, doses of 600 to 1,000 mg/kg applied for 1 - 5 days did result in percutaneous absorption of the insecticide followed by death within the period of application. In contrast to the dog, the rabbit readily absorbed lethal amounts of toxaphene through the skin when the chemical was applied as a solution with dimethyl phthalate, or mineral oil, or when dispersed in water. With the use of a dust, such a species difference was not apparent. Again the liver tissue of sacrificed rabbits showed that degenerative changes had occurred.

There is evidence that in a suitable vehicle toxaphene can be absorbed through the skin of rabbits. The application of 250 mg/kg in peanut oil to the skin caused death to rabbits within 24 hours.

From kerosene solution, 100 mg/kg applied to the skin daily for 6 days gave a high mortality rate in both the rabbit and guinea pig. By contrast, there was poor absorption of toxaphene through the skin when mixed in an inert powder. Rabbits required 500 to 1,000 mg/kg of toxaphene applied as a 40% dust to give symptoms of poisoning or death in 24 hours. This species was unaffected by daily application to the skin for 6 weeks of a 5% toxaphene dust at the rate of 125 mg/kg (95)(Table 13.2).

In the rat, differences in dermal toxicity were observed. Gaines (94) found the dermal LD_{50} in males to be 1,075 mg/kg and females 780 mg/kg.

Johnston and Eden (117)(Table 13-3) dipped rabbits into suspensions of wetable powders of toxaphene to study the topical

Table 13: Summary Of Dermal Toxicity Studies

SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
1. Rabbit	100 mg/kg	Topical	Daily for 30 days	- as 5% dust - no mortality	118 (1949)
	500 mg/kg	Topical	Daily for 5-14 days	- applied as a 50% dust - no mortality	
	800 - 1000 mg/kg	Topical	Daily for 1-5 days	- all animals died when toxaphene applied as a solution in mineral oil or methyl phthalate	
Dog	200 mg/kg	Topical	Daily for 22 days	- applied as 50% dust or in dimethyl phthalate solution; no adverse effect, but at autopsy liver showed degenerative changes similar to those observed following oral administration of toxaphene	
	600 mg/kg	Topical	Daily for 30 days		
2. Guinea pig	5% in kerosene	Oral	Single	- acute oral LD_{50} between 350 - 375 mg/kg	95 (1952)
	5% in corn oil	Oral	Single	- acute oral LD_{50} between 250 - 280 mg/kg	
	100 & 800 ppm	Oral	Daily for 6 weeks	- no changes in growth, blood cell count, urinalysis, reproduction, mortality or histological profile from normal	
	100 mg/kg	Topical	Daily for 6 days	- from kerosene solution caused high mortality	
Rabbit	125 mg/kg	Topical	Daily for 6 weeks	- no effect	
	100 mg/kg	Topical	Daily for 5 days	- from kerosene solution causes high mortality	
	250 mg/kg	Topical	Single	- applied to the skin in peanut oil solution, toxaphene killed the rabbits in 24 hours	
3. Rabbit	372 - 1099 mg/kg	Topical	2 minute dip	- topical LD_{50} (wettable powder in H_2O) between 1025 - 1075 mg/kg - noted loss of appetite, extreme nervousness and convulsions - vein dilation and hemorrhages - livers pale, but kidneys normal	117 (1953)

toxicity of the pesticide. By measuring the change in volume after dipping the animals, the quantity of insecticide applied was estimated. Symptoms of poisoning included loss of appetite, extreme nervousness and convulsions. At autopsy, dilation of the veins and hemorrhages in the intestines were observed and the liver was pale and mottled, but the kidneys appeared normal. A topical LD_{50} between 1,025 and 1,075 mg/kg was calculated.

6.6 Inhalation Toxicity

Almost no information on the toxicity of toxaphene by inhalation was found in the literature. A WHO report gave an LC_{50} for 2 hour exposures to mice as being about 2000 mg/m^3 of air per minute (11). No experimental details were available.

6.7 Biochemical Effects Of Toxaphene

As with other chlorinated hydrocarbon insecticides (133), the administration of toxaphene causes an increase in liver microsomal enzyme activity (49, 50, 119). Through this action, toxaphene can influence the toxicity of drugs and other chemicals detoxified by hepatic microsomal enzymes, as well as affect steroid metabolism because of the increase in liver microsomal enzymes.

6.7.1 Mammalian Systems

6.7.1.1 Rats

The effect of toxaphene on liver microsomal activity was studied by Kinoshita et al. (134), in 1966.

Thirty day old rats were fed toxaphene in their diet at levels of 0.2, 1, 5, 25, or 50 mg/kg (ppm) for 1, 3, 6, or 13 weeks.

The activity of hepatic microsomal enzymes was examined on groups of animals during this period. The oxidative detoxification of O-ethyl-O-(4-nitrophenyl)phenylphosphonoaminopyrine(EPN) was increased after one week of feeding the animals 5 or more mg/kg (estimated to be about 1 mg/kg/day) of toxaphene. There was no effect shown with animals receiving 0.2 or 1.0 mg/kg in their diet. Similarly, although the time frame of changes differed, 0.2 and 1.0 mg/kg had no effect on the induction of O-demethylase activity, whereas 5, 25, and 50 mg/kg all resulted in increases in activities, although probably only the latter two doses were statistically significant. Similar findings were noted for the induction of N-demethylase enzymes, although the effect of 5 mg/kg was greater than with O-demethylase. Quantitative measurements of the amount of enzyme induction occurring with DDT and toxaphene showed that the lowest level that produced an increase in one or more of the enzyme systems was 1 mg/kg for DDT and 5 mg/kg for toxaphene. Toxaphene was less effective than DDT at all the dietary levels tested.

Peakall (135) fed rats toxaphene at one half the LD_{50}(120 mg/kg) and sacrificed the animals at 1, 5, and 15 days. Liver weights and hepatic microsomal enzyme activity were increased at day 5 and 15 and plasma testosterone was significantly decreased at day 15. In a second experiment, rats were fed 2.4 mg/kg daily and sacrificed at 1, 3, and 6 months. Liver weights and enzyme activity were increased compared to controls, but testosterone levels did not differ by the end of the experiment. It was concluded that enhanced hepatic enzyme induction caused only a transient drop in circulating testosterone levels, followed by a return to normal values.

The effect of toxaphene and other halogenated hydrocarbon insecticides on the metabolism of estradiol and estrone by liver enzymes was examined by Welch et al. (119), as well as their effect on the uterotrophic action of these estrogens. Insecticides were injected intraperitoneally at a dose of 25 mg/kg daily for 7 days.

The rats were killed 24 hours after the last dose and the metabolism of ^{14}C-estrone by liver microsomes was found to be almost double compared to corn oil treated control rats. A similar dose of toxaphene resulted in a 71% inhibition in the action of estrone on the rat uterus, as determined by uterine wet weight. The investigators concluded that halogenated hydrocarbon insecticides, including toxaphene, had a stimulatory effect on the *in vitro* metabolism of estrone, which was associated with a decreased action of exogenously administered estrone on the uterus *in vivo*. These results were in accord with observations indicating that treatment of mice or rats with several halogenated hydrocarbon insecticides decreased the concentration of estrogen in the uterus.

Kuzminskaya and Alekhina (136) observed that in rats, a single dose of toxaphene of 120 mg/kg resulted in a decrease in total lactate dehydrogenase (LDH) activity in liver. However, the isoenzyme spectrum of LDH was characterized by an increase in the amounts of LDH-1, LDH-2, and LDH-3 and a decrease in LDH-4. The overall LDH activity was not changed in blood serum. The administration of toxaphene at a dose of 2.4 mg/kg daily (1/100 the LD_{50}) caused a reduction in the overall LDH activity after 6 months. These findings suggested that toxaphene did not result in an increase in lever cell permeability to LDH, but it might have caused an increase in the permeability of other tissues to LDH. The chronic administration of toxaphene resulted in a reduction in serum LDH similar to that seen for liver.

As a result of this study, it was concluded that the introduction of toxaphene into an animal, both as a single large dose and as repeated small doses, produced conditions of hypoxia in the organism and disturbs the normal course of energy metabolism in liver tissue.

6.7.1.2 Cattle

Pardini et al. (137) examined the effect of toxaphene and other pesticides on the electron-transport of mitochondria from beef heart. Toxaphene inhibited both the NADH-oxidase and succinoxidase activity of beef heart mitochondria. These preliminary studies suggested that some chlorinated insecticides altered mitochondrial electron transport in vitro, but further investigations are needed to determine if this enzyme inhibition was in fact a significant factor in the toxicology of the insecticide in the intact organism.

6.7.2 Aquatic Systems

Although no experimental evidence was presented, Sanborn et al. (77) commented that in wild carp, toxaphene appeared to interfere with normal maintenance of skeletal integrity. Mehrle and Mayer (89) published data showing that changes in the amino acid composition and collagen content of the backbone of fish lead to the development of "broken back" syndrome (see section 6.5.3).

Bengtsson (138) discussed the skeletal fragility and fractured vertebrae which occurred in three species of fish following toxaphene treatment. The damage in these instances was suggested to be attributable to a vitamin C deficiency. All the vitamin C that is naturally in the diet of the fish may have been used for the detoxification of toxaphene or other toxic chemicals, so that there was little remaining for bone development.

Other work on the biochemical effects of toxaphene appeared to be concerned with the study of in vitro energy systems, where correlation between ATPase inhibition and the neurotoxic effects of toxaphene were attempted.

Yap et al. (65) investigated the effect of toxaphene on ATPase from the brain of bluegill fish. Oligomycin sensitive and insensitive Mg-ATPase were inhibited 61-77% by toxaphene. The NaK-ATPase was inhibited 44%. The ED_{50} for the mitochondrial Mg-ATPase was 6.9. There was a strong correlation between certain measurements of the neuro-toxic effects and the ATPase inhibitors. The cyclodienes were found to be separable on the basis of longer latency periods for neurotoxic effects and rather unimpressive effects on the ATPase system.

The effect of toxaphene on catfish ATPase from brain, kidney and gill tissues was studied by Desaiah and Koch (139) in order to study the possible mode of action of toxaphene. The pesticide inhibited all three of the ATPases and it appeared that different ATPases had different degrees of sensitivity to the inhibiting effect of toxaphene. It was suggested that the differences could be due to the fact that toxaphene was a mixture of 175 components and each component may have exerted different effects on the various ATPase activities.

Rutschke and Brozio (140) examined the cytotoxic effects of toxaphene in carp hepatic cells treated in vivo with 0.001, 0.01 and 1.0 mg/l toxaphene. At the lowest doses there was condensation of chromatin, mitochondrial swelling and proliferation of rough endoplasmic reticulum (RER). At higher (0.01 to 1.0 mg/l) doses, chromatin condensation was more pronounced, mitochondrial inner structure was destroyed, as was the outer mitochondrial membrane. Disruption of the RER also occurred. Mitochondrial swelling and proliferation of RER probably resulted from the initial stimulation of enzyme synthesis by toxaphene in the initial stages of poisoning.

6.8 Carcinogenicity

The National Cancer Institute (U.S.A.) tested toxaphene on rats and mice, but the data are still unverified and analysis of the data is not complete (January, 1977). A preliminary examination of the NCI studies on rats and mice has indicated that toxaphene may be considered carcinogenic in both these animal species. Also, investigations which were completed in 1969 with strobane, a chemical closely related to toxaphene, indicated an increased incidence of lymphomas and hepatomas in mice fed that chemical. To date, none of these data appear to have been reviewed under the U.S.-E.P.A's interim policy (53). There is a need for further work in this area, particularly in view of the current knowledge of the tumorigenic potential of chlorinated pesticides in man (141).

6.9 Mutagenicity

There were no reports located in the literature on the mutagenicity of toxaphene.

6.10 Reproductive Studies

6.10.1 Rodents

Kennedy et al. (124) reported on the effect of toxaphene on the reproductive performance of 3 generations of Sprague-Dawley rats. Toxaphene, at dietary levels of 25 and 100 mg/kg (ppm), were fed to weanling rats for 79 days before mating. Administration was continued during mating, gestation, weaning of two generations, or for a period of 36-39 weeks. No effect on litter size, pup survival or weanling body weights was observed, and fertility and lactation were also normal.

After 36 weeks of toxaphene, 3 parental generations were subjected to gross and histological examination, where the only changes seen were white cytoplasmic fatty vacuolization in livers of animals receiving the 100 mg/kg diet. This change was not observed in animals receiving 25 mg/kg in the diet and was not accompanied by any adverse effect on growth, mortality, clinical parameters, organ weights or reproductive capacity. No effect levels were judged to be 25 mg/kg diet (1.4 mg toxaphene per kg bodyweight per day)* for toxaphene and reproduction was not not affected at levels of 100 mg/kg diet.

* see footnote section 6.4.4.1

Similarly, little or no effect was noted, in an abstract by Keplinger et al. (125), through 5 or 6 generations of mice fed toxaphene at 25 mg/kg (ppm). It was suggested that fertility, gestation, viability, lactation and survival were monitored.

Chernoff and Carver (142) found that rats, which received 35 mg/kg of toxaphene by intubation per day, during the period of organogenesis on days 7-16 of gestation, had a 31% maternal mortality rate. Mice on the same dose had an 8% mortality. There was a dose-related reduction in the weight gain of the treated animals for both rats and mice during the course of the study, although the statistical significance of the weight gains is questionable. No dose related changes were noted in fetal mortality in rats and there were no significant dose-related responses in the fetal mortality or weight of mice from treated litters.

6.10.2 Birds

Toxaphene was fed in the diet for 24 weeks to white Leghorn layers at concentrations of 0, 10, or 100 mg/kg (ppm). Arscott et al. (121) reported that egg production was lower for the 100 mg/kg (ppm) group than for controls. It should be noted that the control group had a production success of 68.5%; 10 mg/kg, 74.6%; and 100 mg/kg, 61.5%; so that the differences may not be highly significant. No adverse effects on various performance parameters, including fertility, hatchability and survival of progeny, were observed.

Egg production in female pheasants (Phasianus colchious) was depressed significantly in pheasants fed 300 mg/kg (ppm) of toxaphene in their diet (122), but the laying rate (eggs per day) did not differ in birds receiving 100 mg/kg/day in their mash. Both these dose levels of toxaphene were previously established as sub-lethal to the adult birds. Egg fertility and the hatchability of eggs from birds receiving the 300 mg/

kg (ppm) dose were significantly lower than for the controls over the 8 week study period. The lowered hatchability was associated with the poor condition of the adult females, rather than with the concentration of the insecticide within the eggs. None of the parameters examined were significantly affected by the feeding of 100 mg/kg of toxaphene in the feed. It was calculated that the average daily consumption of toxaphene per bird per day was 4.74 mg for those receiving 100 mg/kg diet, and 9.12 mg for those receiving 300 mg/kg of toxaphene.

No reproductive impairment occurred in Mallards, receiving a a long-term dietary dosage of 7 mg/kg (ppm), and Starlings tolerated 45 mg/kg for extended periods (cited in 76).

6.11 Teratogenesis

Only two reports were reviewed relative to the potential teratogenic effects of toxaphene. Oral administration of 35, 25 or 15 mg/kg on days 7-16 of gestation, to mice and rats, resulted in no anomalies in newborn rats, but five litters of mice which received the highest dose of toxaphene had one or more individuals with encephaloceles. The degree of skeletal ossification was decreased in rats but not in mice. It was concluded that the administration of toxaphene to rats and mice during the period of embryonic organogenesis resulted in some fetotoxic effects at levels which caused maternal toxicity.(142)

The study of Kennedy et al. (124)(see section 6.9 for details), in which three generations (6 litters) of rats were fed 25 or 100 mg/kg (ppm) toxaphene in their diet, showed no evidence of any abnormal anatomical structures in offspring. All progeny were viable and normal in size.

6.12 Interactions And Synergistic Effects

6.12.1 Insects

Certain chlorinated terpenes, such as 2,6-dichlorocamphene, are synergistic with DDT. The discovery of the synergistic action between toxaphene and DDT, when in a 2:1 ratio, led to a number of insecticide formulations of this type for use where resistance to one or the other of the constituents had developed. Thus, an emulsifiable concentrate, containing 4 pounds of toxaphene and 2 pounds of DDT per gallon, proved to be highly effective against the cotton boll weevil in unusually difficult situations. In some instances, methylparathion was added to give rapid knock-down of boll weevils, and in other instances, parathion was included for aphid control. Dust formulations incorporating the 2:1 ratio have also been used on cotton and have found certain applications on vegetables. The co-formulation of toxaphene with parathion combines the activity spectrum of both toxicants and is effective by oral absorption, contact and fumigant action against a diversity of lepidopterous larvae, leaf miners, aphids, scales, and other pests, some of which are unaffected by toxaphene alone. Limited recourse has also been made to combinations with various miticides, fungicides (maneb, zineb), organophosphorous insecticides (dioxathion, diazinon, malathion, etc), endrin, endosulfan, and Bacillus thuringiensis for specific purposes. In some cases, co-formulation of toxaphene with other materials has caused varying degrees of phytotoxicity, or the residual toxic effect of the organochlorine has been significantly reduced (2).

In adult lady beetles, toxaphene, at a dose of 10-60 ug/insect, decreased longevity and prevented oviposition. Atallah and Newsom (103) also reported that active beetles were more sensitive to the insecticide than were diapausing beetles. For example, almost 50% of the active beetles died within 7 days after treatment with 10 ug/insect, whereas only 20% of diapausing beetles died following the same dose. Various 2:1 mixtures of toxaphene and DDT exhibited synergistic action as measured by decreasing longevity and reduced reproduction. The toxaphene LD_{50} was 21.8 ug/active beetle and 44.2 ug/ diapausing beetle. For the toxaphene-DDT mixture, the LD_{50} was 7.1 - 3.6 ug/active beetle (584 mg of toxaphene and 292 mg of DDT per kilogram).

Adkisson (105) studied the development of resistance by the Texas tobacco budworm (Heliothis virescens) to combinations of toxaphene with DDT during 1963-65. During this time, the LD_{50} for toxaphene plus DDT increased from 0.5 to 3.52 mg of insecticide per gram of larvae. These values indicated increasing resistance of the tobacco budworm of approximately 6-fold to toxaphene plus DDT.

Data were published by McGarr and Wolfenberger (106) on the effectiveness of toxaphene alone, and in combination with other insecticides, in the control of cotton insects, such as the boll worm and boll weevil. In general, the combination of the insecticides was more effective than toxaphene alone.

Wolfenbarger (104) also determined the LD_{50} of toxaphene to the tobacco budworm and found it to be, on average, about 5 mg/g. It was also shown that a 1:1 mixture of toxaphene and DDT had a synergistic effect, with a resulting LD_{50} of 0.5 mg/g. A maximum dose of 200 ug of toxaphene per larva of boll worm was not lethal and no LD_{50} was determined. However, again a combination of toxaphene and DDT proved to be synergistic and an LD_{50} of 0.14 was calculated for a 1:1 mixture. DDT alone had an LD_{50} of 2.5 mg/g.

6.12.2 Rodents

Toxaphene, in combination with other chlorinated hydrocarbons, carbamates or organophosphorous insecticides has also proven to be more toxic to rats and mice, as well as insects.

Keplinger and Deichmann (143) studied the acute oral toxicity (LD_{50}) of equitoxic doses of combinations of 15 pesticides in rats and mice. The expected LD_{50} of a mixture was compared with the observed or actual LD_{50} and expressed as a ratio. In most instances, toxaphene had an additive effect with other pesticides; including aldrin, dieldrin, chlordane, DDT, malathion, carbaryl, V-C13, delnav, parathion, diazinon, endrin, aramite and methoxychlor. Some degree of antagonism was shown between toxaphene and trithion, and diazinon and parathion. The ratio of expected to be observed LD_{50}'s were between 0.5 and 0.7, indicating that less than an additive toxicity occurred.

Potentiation of toxicity was observed in mice fed a mixture of toxaphene, chlordane and malathion. The ratio of expected to observed LD_{50} was 1.57. Toxaphene toxicity was not potentiated in rats administered malathion or chlordane concurrently. The three compounds were not listed together in rats.

6.12.3 Fish

In a single study with fish, Macek (144) found that toxaphene, in combination with malathion, DDT or parathion, had additive effects when tested for toxicity against bluegill fish. Concentrations of toxaphene used in these various pesticide combinations ranged from 5.5 to 27 ug/l.

7. HUMAN HEALTH EFFECTS

Newsom (75) reviewed the biological significance of long-term human exposure to insecticides in the environment and pointed out that it was little better understood at the time of his writing (1966) than it was 10 years previously. He expressed the opinion that the conviction was growing that the delayed effects of long-term exposure may never be known. The difficulty of species to species extrapolation was such that questions relating to human toxicity and hazard must be found in human epidemiology or by controlled studies in man. He also pointed out that, even if controlled studies in man were possible, lack of suitable unexposed control groups could present a serious problem. He drew attention to the fact that human exposure to residues of DDT for almost a generation indicated no hazard at the concentration involved. It was reassuring to Newsom that the chlorinated hydrocarbon insecticides apparently conformed to the general principle of pharmacology, in that a steady state of storage was reached with tolerated intake of a chemical. At tolerated dosages, man approached storage equilibrium of DDT in about 1 year. Apparently, DDT stored in the population in the United States reached a "plateau" at about 12 mg/kg (ppm) by 1951, and remained unchanged at the time of his publication.

As would be expected, there are few controlled studies of the effect of toxaphene in man. There have, however, been a few reports of human toxicity which are reviewed in section 7.2.

7.1 Metabolism

No quantitative or controlled studies on the absorption and distribution of toxaphene in man appeared to be available in the literature. On the basis of the range of toxicity noted in animals, it is assumed that toxaphene absorption will also be irregular. Based on animal studies, it can be expected that toxaphene will be absorbed by man from the alimentary tract, through the respiratory system and through the skin. In addition, it is likely that the physical state of the product and the vehicles used will influence the pesticides absorption.

Although little direct information is available, most toxicology texts point out that intestinal absorption of toxaphene is increased by the presence of digestible oils, and liquid preparations (oil solvents) penetrate the skin far more readily than do dusts or powders (95, 117, 118). At least 8 human fatalities have occurred as a result of exposure to toxaphene (95, 145, 146).

Little is known about the distribution, storage, detoxification, and excretion of the compound by man. The lack of suitable microanalytical chemical methods for the positive identification of minute quantities of toxaphene in tissues or body fluid has prevented an accurate determination of its metabolic fate. The high lipoid solubility possessed by toxaphene suggests that it is probably distributed throughout the various fat depots of the body, but not to as great an extent as other chlorinated pesticides and organophosphorous compounds.

Toxaphene is believed to be slowly detoxified in the liver. This assumption was based on 2 observed characteristics; namely, its close chemical relationship to camphor, which is known to be detoxified in the liver, and the isolation of ethereal sulphate and glucuronic acid conjugates of toxaphene in the urine. Toxaphene was also found to be excreted in mothers' milk (49).

Harfield (147) reported toxaphene to be absorbed through the skin and that 2.4 g applied daily to the skin was dangerous.

7.2 Acute Toxicity

Although not definitely ascertained, the lethal oral dose for an adult is estimated to be 2 - 7 grams, representing a toxicity about 4 times that of DDT (148).

Toxic symptoms in acute poisoning usually appear within 1 hour, and death occurs within 4 to 8 hours. The onset of symptoms is characteristically abrupt. Generalized convulsions constitute the only definitive manifestation of the intoxication, but cyanosis has appeared before convulsions in at least 1 case (146).

In adequate dosage, toxaphene interferes with axonic transmission of nerve impulses and, therefore, disrupts the function of the nervous system, principally that of the brain. This results in behavioural changes, sensory and equilibrium disturbances, involuntary muscle activity, and depression of vital centers, particularly that controlling respiration. Adequate doses increase the irritability of the myocardium and cause degenerative changes in the liver.

Frequent symptoms and signs of poisoning which occur following toxaphene ingestion include apprehension, excitability, dizziness, headache, disorientation, weakness, paresthesia' muscle twitching, tremor, tonic and clonic convulsions (often epileptiform), coma. Soon after ingestion, nausea and vomiting are often prominent. When chemicals are absorbed by parenteral routes, apprehension, twitching, tremors and convulsions may be the first symptoms. Respiratory depression is caused by the pesticide and by the petroleum solvents in which toxaphene is usually dissolved. Pallor occurs in moderate to severe poisoning. Cyanosis may result as convulsive activity interferes with respiration (149).

A partial summary of the dose-response data presently available for humans in the literature and the expected lethal concentration range of toxaphene for humans is given in Table 14.

In an unpublished report (cited in 69) Shelavski exposed 25 human volunteers in a closed chamber to an aerosol of toxaphene for 30 minutes a day for 10 consecutive days at an average concentration of 500 mg/m^3 of air. After 3 weeks they received the same exposure on 3 consecutive days. Based upon an assumed retention of 50% of the inhaled toxaphene, each individual received a dose of 75 mg daily or about 1 mg/kg per day. Complete physical and laboratory examinations failed to reveal any

Table 14: Summary Of Human Toxicology Data

	SPECIES	DOSE	ROUTE OF ADMINISTRATION	LENGTH OF ADMINISTRATION	EFFECT MEASURED AND/OR OBSERVATIONS	REFERENCE (YEAR)
1.	Human	2 - 6 g/kg	Oral	Single	- estimated fatal oral dose to man	(1949)
		2.4 g	Dermal	Daily	- considered a dangerous dose to man	
2.	Human	9.5 - 47 mg/kg	Oral	Single	- accidental poisoning - vomiting but no convulsions noted - no deaths among 3 victims	95 (1952)
3.	Human	<10 mg/kg	Oral	Single	- less than 10 mg/kg can cause convulsions	145 (1967)
4.	Human	46 g	Dermal	Single	- very dangerous dose	69 (1975)
		10 mg/kg	Oral	Single	- this dose or less can lead to convulsions in some with no effect in others	
		1 mg/kg	Inhalation	Per day for 10 days	- complete physical and laboratory examination failed to reveal any toxic manifestations	
5.	Human	2 - 7 gm	Oral	Single	- considered sufficient to cause death in adults (about 4 times as toxic as DDT)	1 (1976)
6.	Human	60 mg/kg	Oral	Single	- estimated human fatal dose - continued ingestion of small doses may cause liver damage	Merck Index

toxic manifestations.

McGee et al. (95) noted in 1952 that during a 5 year period in which chemists had been manufacturing toxaphene, there had not been an instance of poisoning reported in man. Periodic examination of workers showed no alteration in reflexes, subjective manifestations and disorders of the nervous system, or other signs of health impairment. Urinalysis and blood cell counts were normal; furthermore, they had learned of no instances of accidental poisoning among exposed workers in the manufacture of insecticides containing toxaphene.

They did, however, report on three deaths in children presumably resulting from the accidental ingestion of toxaphene. A fourth child was also poisoned but recovered. None of the cases provided any useful information on the quantity of toxaphene ingested which resulted in death. In one instance it was thought that a 17-month old child ate about half a teaspoon of full strength toxaphene (probably 60% concentrated in special solvents).

Two instances of group poisoning were reported, one in which a collard patch had been sprayed with toxaphene-containing insecticide. It was found that even though the greens had been washed three times, the samples contained over 3,000 mg/kg (ppm) of toxaphene. In these instances, all of the affected people survived. The estimated dose of toxaphene in the three instances reported was 9.5 to 47 mg/kg of body-weight.

A second instance of group poisoning occurred from eating of garden chard which had been sprayed with an unspecified amount of toxaphene. Again, no deaths were reported. No estimates of intake were provided.

In both the above instances the description of the toxic symptoms confirmed laboratory findings that toxaphene acts chiefly as a convulsant drug (95). The onset of poisoning was characteristically abrupt and not associated with abdominal pain, involuntary vomiting or diarrhea. Illness was manifested by a skeletal muscle response to central nervous system stimulation and by loss of consciousness. The cardinal symptom of toxaphene intoxication was convulsions, and the use of barbituates to counter the acute central nervous system stimulation was recommended.

Hercules, a major producer of toxaphene, in a news item in Chemical Week (May 11, 1977), claimed to have surveyed their workers in its plants over the past 28 years and found no increase in tumor incidence or other diseases over those found in the general population.

In 1953, a case of a 16-month-old child ingesting a fatal dose of toxaphene was reported (146). Although no indication of the quantity ingested was given, the course of the patient's demise was described by the attending physician and the question of the dose of barbituates required to control the convulsions was raised.

The case of a 70-year-old man, who developed symptoms of poisoning through the cutaneous absorption of a toxaphene-lindane mixture, was recorded by Pollock in 1958. Because the symptoms of the two drugs were practically identical, it was impossible to differentiate between the two as to the major cause of toxicity. The patient went through symptoms similar to that for oral ingestion, including nausea and vomiting, eventually going into a semi-comatose condition with temperature fluctuations and dehydration. Nausea, vomiting and epigastric distress did not stop until the 10th day after exposure, and the dullness of sensorium was replaced by normal reaction at that time.

Warraki (150), in 1963, reported the case history of two field workers who were exposed to toxaphene and presumably had a toxic reaction to the chemical. It was impossible to determine the quantity to which the workers were exposed and both workers survived. However, the authors provided a summary of the clinical features of toxaphene poisoning. The symptoms were mild, with a slightly elevated sedimentation rate, compared to extensive radiological findings of miliary shadows in both lungs. Their general condition was good and no toxemia was observed. The presence of sudden exertional dyspnea, tachycardia and weakness, together with a low blood pressure, led to the suspicion of a cardiac attack. The presence of blood eosinophilia and high serum globulin were also recorded. The clinicians noted a dramatic clinical reversal and radiological response as early as three weeks after beginning treatment. Complete reversibility of the x-ray shadows, symptoms and physical signs returned to normal on treatment with cortisone plus anti-tuberculosis drugs.

A 9-month old infant ingested an unknown quantity of a 1:2 mixture of DDT and toxaphene, which resulted in various symptoms such as moist skin, myosis, diarrhea and dyspnea (145). Seizures were temporarily controlled with sodium phenobarbital, but convulsions, respiratory arrest and death followed. Autopsy revealed only cerebral edema. It was found that the ratio of toxaphene to DDT in brain and liver tissue was 10:1 and in kidney 3:1. Levels in brain were estimated to be as high as 14 mg/kg (ppm), whereas the DDT levels were 1.55 mg/kg (ppm). Because of the relatively high tissue levels of toxaphene, the authors suggested that toxaphene was more important than DDT as the cause of death. It was speculated that toxaphene was more readily absorbed than DDT and possibly more readily concentrated in the brain and liver. These authors commented that it had been estimated that a dosage of 10 mg/kg, or even less, of toxaphene could lead to convulsions.

Yoder et al. (151) provided evidence that workers who were in heavy contact with pesticides, including toxaphene, showed a marked increase in the frequency of chromatid lesions. Unfortunately, workers could not be examined who were exposed to a single agent, and in most instances exposure to toxaphene also included exposure to other organic chlorine insecticides. The extent of visible chromosomal damage in lymphocyte cultures prepared from the exposed groups was considerably greater during the heavy insecticide spraying season. The investigators suggest there was a need to investigate further the potential mutagenicity of these compounds.

Patch testing of 200 unselected human subjects by Parker and Beacher (152) showed that toxaphene was neither a skin irritant nor a skin sensitizer.

7.3 Clinical Effects And Treatment Of Overdose

Toxaphene is a general convulsant which acts on the central nervous system. It causes diffuse stimulation of the brain and spinal cord resulting in generalized convulsions of a tetanic or clonic character. In this respect, it acts somewhat the same as DDT and certain other chlorinated hydrocarbon insecticides. Since toxaphene is a chlorinated camphene, it is suspected to have the medullary excitant effects of camphor. Such effects have been observed in several species of laboratory animals.

Salivation is usually the first sign of toxicity in animals. This is followed by emesis, which is believed to be caused by direct stimulation of the emetic centre. Shortly after the onset of vomiting, intermittent muscle spasms develop. These are followed by generalized convulsions at 3 to 5 minute intervals. Convulsions become progressively more frequent until finally a convulsive state exists which terminates in exhaustion and death from respiratory failure. The onset of symptoms usually occurs in 1 hour, although symptoms may be delayed as long as 3 or more hours after an oral toxic dose. Death occurs in 4 to 8 hours or may be deferred up to 24 hours.

The occurrence of salivation, vomiting and reflex excitability to sound suggests a generalized stimulation of medullary centers. Stimulation of the vasomotor center, evidenced by the abrupt rise in blood pressure in the presence of complete muscular paralysis, occurs in curarized animals following intravenous doses of the compound. There are, however, no significant changes in mean arterial blood pressure following oral administration of toxaphene. This difference in response is probably accounted for on the basis of incomplete absorption of the toxicant from the gastrointestinal tract. The generalized epileptiform spasms which terminate in respiratory failure are also characteristic of a camphor-like compound.

The treatment of toxaphene poisoning is directed toward the evacuation of the stomach and bowels, and control of convulsions and other central nervous system manifestations. Gastric lavage and saline carthartics are the accepted measures for the removal of ingested insecticide. Toxaphene-induced convulsions in experimental animals seemed to be controlled with greater ease when this practice was followed. This was probably because of a great affinity for lipoid substances and the negligible water solubility which is characteristic of toxaphene and other chlorinated bydrocarbon insecticides. Oily carthartics and fatty substances are usually contraindicated because of their capacity to increase the absorption of the toxicant. Prompt removal of the oil-solubilized insecticide before passage into the intestines, however, reduces the chance for absorption of appreciable amounts of the toxicant (148).

The U.S. Environmental Protection Agency has published a recommended treatment for poisoning (149). It was essentially the same for all chlorinated pesticides and consists of gastric lavage or emptying, supportive ventilation and administration of drugs such as diazepam to control convulsions. It has also been suggested that, after gastric emptying, the administration of activated charcoal may inhibit further absorption of any remaining toxaphene. General supportive treatment and good diet was recommended.

7.4 Epidemiology

The only study of human tissue levels of toxaphene was published by Kadis et al. (153). Autopsy specimens from up to 50 cases were sampled in 1967-1968 at the University Hospital at Edmonton, Alberta. It was reported that in spite of the fact that toxaphene had been used extensively in agricultural and public health programs in Alberta, residues were not detected in the 217 tissues analyzed.

8. EXPOSURE, DOSE ASPECTS

8.1 Dose-Effect And Dose-Response Relationships

By extrapolation from animal studies and the few human poisoning reports available, a single oral dose of 2-7 g is probably sufficient to cause death (1). Using the classification from Clinical Toxicology of Commercial Products (1), this would place toxaphene in the class of slightly to moderately toxic compounds. However, the publication itself classifies toxaphene as having a toxicity rating of 4, or very toxic, with the probable oral lethal dose being between 0.5 and 5 g/kg.

No quantitative data were available which permitted estimates to be made of the dose of toxaphene to which humans can be exposed without developing signs of toxicity, nor was any information found which would permit a reasonable estimate of the minimum intake, on a chronic basis, which would lead to liver damage or enlargement.

For convenience, a summary of some of the more relevant dose-effect findings discussed in section 6 is given below.

8.1.1 Body Weight

In general, low doses of toxaphene to mammals did not appear to significantly affect growth. It was reported that in the rat, feeding a diet containing 1500 mg/kg of toxaphene (75 mg/kg/day) for 20 weeks, resulted in treated animals having reduced body weights compared to controls. No quantitative information on the differences was available.

8.1.2 Effects On Liver

In rats, no-effect, or minimal effect levels for changes in liver structure have been estimated to be 25 mg/kg (1.4 mg/kg/day) in the diet, even when fed for several weeks.

Feeding a diet containing 50 mg/kg (2.8 mg/kg/day) to rats lead to slight liver cell changes, including enlargement of centrilobular hepatic cells and peripheral migration of granules. These changes were pronounced in animals receiving about 6 mg/kg/day. In the dog, intake of 3-6 mg/kg/day for 2 years lead to an increase in liver, and in the liver to body-weight ratio.

8.1.3 Effects On The CNS

Dogs receiving 5 mg/kg/day had occasional convulsions after continued toxaphene administration.

Convulsions were reported in rats receiving a diet containing 1000 mg toxaphene/kg (57 mg/kg/day), after 7-10 months.

8.1.4 Effects On The Kidneys

Degenerative changes in renal cells were reported in dogs receiving 5 mg/kg for a few months.

8.1.5 Effects On Biochemical Parameters

Toxaphene was shown to cause changes in hepatic and serum enzyme activities in various mammalian species. These were associated with changes in the ultrastructure of the liver, especially the endoplasmic reticulum. In young rats receiving a diet with 5 mg/kg (1mg/kg/day)* toxaphene for 1 week or more, minimal increases in liver O- and N-demethylase activity were observed. Higher doses (5 mg/kg/day) resulted in statistically significant changes in enzyme activity.

8.1.6 Reproduction and Teratogenesis

In one feeding study, rats ingesting a diet containing 100 mg/kg (about 6 mg/kg/day)* showed no reproductive effects. However, increases in maternal death were recorded in another investigation, when a diet containing 35 mg/kg toxaphene (2 mg/kg/day)* was provided during days 7-16 of gestation.

No teratogenicity resulted from feeding rats a diet containing toxaphene at 100 mg/kg (6 mg/kg/day).

* see footnote section 6.4.4.1

8.2 Calculated Or Estimated Recommended Acceptable Or Tolerable Doses From Available Data

Insufficient human data were available which would permit reasonable estimates of a maximum allowable daily intake (ADI).

There were, in fact, few animal experiments which provided information on the dose causing minimal or no effect on tissue biochemistry and/or pathology.

The author of this document holds the view that significant consideration should be given to investigations which permit quantitative relationships to be realized between dose and histopathological changes. In addition, however, the dose causing more subtle alterations in organ or cell function, such as changes in the rate of detoxification of compounds, induction of enzymes or structural abnormalities in cell organelle architecture and function should also be given considerable weight. Although carcinogenicity and similar chronic, toxic manifestations, such as liver necrosis, are of paramount importance, the objective is to set environmental standards which lead to intakes of a substance which have no adverse effect on the organism. Because the long term significance of many cellular and/or biochemical changes which can occur are not fully understood, it would seem reasonable to calculate ADI's based on doses which cause no effects in man; or in instances where human data is insufficient, in animals. This view would appear to agree with the definition of toxicity stated by the Food Protection Committee of the National Academy of Sciences (U.S.A.), which states that toxicity is the capacity to produce injury. The term is comprehensive and includes the capacity to injure any of the mechanisms of the body, which includes the induction of carcinogenesis (154).

Although it is realized that the validity of extrapolating animal dose-response data to predict safety in man is questionable (154), and may lead to false conclusions or unnecessary legislative restrictions, it is of some interest to estimate a "safe" dose in man based on animal experimentation. Weil (154) presented a rationale in support of using a factor of 1/5000 of a minimum-effect level to calculate the maximum allowable amount for the human. Depending on the biological response measured, this factor may be reduced 10 to 50 fold. Others have suggested factors as great as 100,000,000 be used to define "virtual safety" (155).

The U.S. Environmental Protection Agency (45) has suggested that to determine a "safe" exposure level for man, a factor of 10 may be used if data are from human studies of more than two months duration at a no-effect dose. Alternately, a factor of 100 was recommended when human data were available for study periods of 2 months or less. The Environmental Protection Agency suggested that a factor of 100 be applied to animal data when adequate human information was available to corroborate the animal experiments. When no suitable human data were available, a factor of 500 was "generally" applied to the lowest, long-term dose having minimal or no effect.

The difficulty of estimating "safe" doses in man based on animal dose-response relationships is increased when experimental findings are variable, particularly when they occur for unexplainable reasons. Such is the case with toxaphene, although it should be noted that similar variations have been reported for DDT (49, 50).

For example, Ortega (123) fed rats a diet containing 50 mg/kg toxaphene (estimated to be 2.5-3.0 mg/kg/day) and found liver cell abnormalities after 6-9 months. Treon (69), on the other hand, found no effect at a dietary level of 100 mg/kg (about 5.0-6.0 mg/kg/day). This same dietary dose (100 mg/kg) resulted in significant hepatic histological changes in the study of Fitzhugh and Nelson (132). These investigators reported that 25 mg/kg in the diet (estimated to be 1.25 mg/kg/day) had minimal effects on liver pathology.

As part of a reproductive study (124) it was found that 100 mg/kg of toxaphene in the diet caused changes in liver structure, whereas 25 mg/kg had no effect after 39-42 weeks of administration.

Lehman (131) summarized the effect of toxaphene studies in 1952. He stated that the lowest dietary level which produced gross liver enlargement was 400 mg/kg, and 100 mg/kg was the highest dose which did not cause a gross effect. The lowest dose which produced tissue damage was 100 mg/kg, and 25 mg/kg was the highest level which caused no tissue damage. These facts do not appear to have changed since Lehman's dissertation.

In dogs, the Kettering study (69) found that animals receiving 3.12 - 6.56 mg/kg/day (200 mg/kg on a dry diet basis) showed liver weight increases and hepatic cell enlargement, as well as other changes. One out of three dogs receiving 0.60-1.47 mg/kg/day (equivalent to 40 mg/kg on a dry diet basis) originally was reported to have slight liver enlargement, but re-examination of tissues failed to confirm the original finding. No evidence of toxicity was observed in a feeding study carried out by Industrial Bio-Test Laboratories, and reported in brief in a WHO/FAO publication (69), using dietary levels of 5, 10, and 20 mg/kg (ppm) for up to 24 months.

The fact that in these studies no experimental details were available, such as body-weight and food consumption, makes it difficult to properly assess the findings.

In a U.S. Environmental Protection Agency criteria document (76), reference was made to a report by Lehman, in 1965, which could not be located by the reviewer. In this report it was supposedly stated that the highest level of toxaphene found to have minimal or no long-term effects in dogs was 10 mg/kg in the diet, or 1.7 mg/kg of body-weight/day. This figure was, as is discussed in section 8.5, used to calculate a permissible level of 5 ug/l of toxaphene for domestic water supplies in the U.S.A. The author does not know why the value of 1.25 mg/kg/day, as reported by Fitzhugh and Nelson for the rat (131), was not employed.

In another Environmental Protection Agency publication (45), the lowest long-term levels of toxaphene, with minimal or no effects, were reported as 1.7 mg/kg/day in rats (10 mg/kg in the diet) and 8.0 mg/kg/day in dogs (400 mg/kg in the diet). This led to the calculation of a maximum allowable limit (MAL) of 0.025 mg/l in drinking water (a safety factor of 500 was used). However, for organoleptic reasons, the recommended limit was 0.005 mg/l.

Of interest in this latter report was the fact that the recommended limit for toxaphene was based on estimates in which the average weight of animals was 300g, with an average daily dietary intake of 50 g. These figures differ from those given in The Toxic Substances List (NIOSH, 1974), which are 200 g for adult female and 250 for male rats, with both sexes having food consumptions of 15 g/day. The Canadian Council of Animal Care suggests the average weight of adult male rats to be 400 g and females, 250 g, with both consuming 20 g of diet/day, on average. Figures approximating the latter values were used where necessary in this document.

In view of the lack of detailed experimental conditions, which would permit proper scientific evaluation of the toxicological data available, it is difficult to find a value, from animal experimentation to use in estimating an allowable daily intake.

In the present review of the literature, the lowest dose of toxaphene which resulted in a measurable change in liver biochemistry was 1.0-1.3 mg/kg/day (estimated)(134). A diet containing 5 mg/kg was fed to young rats (30 days old with an estimated body-weight of 60-75 g and food intake of 15 g/day). Within 1 week increases in liver O-demethylase and N-demethylase activities were observed. This value was similar to that of Fitzhugh and Nelson of 1.25 mg/kg/day in which minimal pathology of rat liver was reported (131).

Using this latter value, and an arbitrary safety factor of 1,000 (two times that used by the U.S.-E.P.A.), a calculated maximum safe intake for an average 70 kg human adult would be 0.00125 mg/kg/day or 0.09 mg toxaphene/day.

Because toxaphene is not in general use or manufactured in Canada, its ingestion by way of foods, inhalation of air or water, probably approaches zero. Any toxaphene which might be consumed would be from imported foods, which would probably represent a negligible intake. Thus, consideration should be given to whether there is a need to set a standard for Canadian drinking water supplies. It could be argued that a standard should be set in order to have one available for those occasions when toxaphene is used for special purposes. However, this may not be sufficiently frequent to necessitate the setting of limits in drinking water.

8.3 Current Exposures From Food, Air And Water

As was discussed in section 5, in view of the fact that, except in rare instances, the Canadian food and water supplies contained no detectable toxaphene, it is likely that the intake of this pesticide by the average Canadian is virtually zero.

It is unlikely that any toxaphene would be ingested from the atmosphere as the pesticide is not used extensively or manufactured in Canada, and ambient air levels of the pesticide are probably such as to be non-detectable.

8.4 Special Risk Groups

Special risk groups include persons associated with the manufacture, use, and research into the safety of toxaphene (11).

In view of the potential hepatotoxic effects of toxaphene it is probable that individuals with diminished liver function, or hepatic necrosis due to drugs and/or other chemicals, or disease states, could be particularly responsive to the toxic properties of toxaphene.

8.5 Published Figures From Other Organizations

The W.H.O., in 1975, recommended a TLV of 0.5 mg/m^3 for workers exposed to toxaphene in manufacturing plants (11). This value is the same as the U.S. - A.C.G.I.H. figure.

Maximum food residue limits have been established by the Joint FAO/WHO Meeting On Pesticide Residues. As these are subject to change at annual reviews, the latest data will be found in the report of the 1973 Joint FAO/WHO Meeting On Pesticide Residues (11).

The Canadian Food and Drug Regulations provide for permissible levels in foods as follows: 3 mg/kg in oats, pineapple, rye and wheat; 5 mg/kg in barley, grain sorghum or rice; 7 mg/kg in beans, blackeyed peas, broccoli, brussel sprouts, cabbage, cauliflower, celery, citrus fruits, egg-plants, fat of meat (cattle, goats, hogs, sheep), lettuce, strawberries, tomatoes, pears, onions and okra.

In Germany, the permissible tolerances are as follows: 0.4 mg/kg in pears, strawberries, raspberries, cherries and plums; 0.04 mg/kg in other plant products.

The Netherlands set tolerance figures for fruit and vegetables, except potatoes, as being 0.4 mg/kg.

In the United States, the acceptable tolerance level in soybeans is 2 mg/kg, and in pineapples and bananas, 0.3 mg/kg in the pulp. In wheat, barley, rye, rice, cottonseed, stone and pome fruit, cane fruit, corn, leafy, fleshy and fruit vegetables, nuts, beans, peanuts, peas, strawberries and fat of beef, sheep, goats, swine and horses, it is 7 mg/kg.

The U.S. - F.D.A. seizure level for fish to be consumed by humans is 5 ug/g fish (53).

The U.S. - E.P.A. has recommended an acceptable limit of 5 ug/l in domestic water supplies based on health effects. However, in order to protect aquatic organisms, a level of 0.005 ug/l was suggested for freshwater and marine life (76, 102).

In deriving the above criteria, the U.S. - E.P.A. used the following rationale (76). They considered the dog to be the most sensitive mammal tested, and that a dose of 10 mg/kg in the diet or 1.7 mg/kg/day was the dose which caused minimal or no long-term effects. (This point is questionable as was discussed in section 8.2). A safety factor of 500 was used on the basis that where no adequate human data are available to corroborate findings in animals, then the total "safe" intake was assumed to be 1/500 of the "no-effect" or "minimal effect" level reported for the most sensitive animal tested.

Using these data, and including the assumptions that 20% of the total intake of toxaphene was from drinking water, and that the average 70 kg person consumes 2 litres of water per day, the criteria was calculated to be 0.024 mg/l (1.7 mg/kg/ day x 0.2 x 70 kg x 1/500 x $\frac{1}{2}$ = 0.024 mg/l).

However, an unacceptable organoleptic effect has been shown to occur with toxaphene, and the lowest acceptable level was determined to be 0.005 mg/l. Therefore, the criterion was set at 0.005 mg/l or 5 ug/l.

In 1968, a tentative Canadian drinking water standard was issued for toxaphene. The objective and acceptable limits were "none detectable", but the maximum permissible level was given as 0.005 mg/l (5 ug/l).

No maximum acceptable daily intake was set by the FAO/WHO Joint Committee On Pesticides (69). That group were of the opinion that although adequate work had been done on the original compound of known composition, the substance presently in agricultural use did not necessarily conform to the specifications of the original material tested biologically (69). They concluded that before an evaluation could be made, the identity of the compounds in use must be established, and future toxicological work must be related to them.

The reasoning behind this statement is difficult for the present author to understand. To his knowledge, the composition of toxaphene has never been delineated and if the presently available product is manufactured using the same toxaphene synthetic process as originally used, then differences between the present and original materials should be minimal and the biological data gathered to date should have sufficient relevance to both to permit setting of intake standards.

As is pointed out in section 9 on future research needs, more information is required on the batch to batch consistency of the composition of toxaphene before truly reliable toxicological data can be generated.

9. RECOMMENDATIONS FOR FURTHER RESEARCH

It appears that a great deal of additional research is required to adequately define the chemical composition and toxicity of toxaphene before truly scientific estimates of the long-term risks of low level intakes can be calculated.

For example, further study is needed on the batch to batch chemical uniformity of the technical product; the variability in biological activity batch to batch; and differences in the chemical make-up of product from different sources. Also, examination of the criteria for control of the degree of chlorination and its relationship to efficacy and toxicity of the pesticide should be carried out.

It would be useful to obtain more information on the nature of the residues in plants, animals, and in water, including investigating the possibility of the formation of photo-oxidation products.

Additional residue data, including levels in air and water, should be gathered in Canada to ensure that toxaphene is not present at significant levels in the environment.

There is a great need to carry out properly designed and controlled low dose, life-time feeding studies in at least 2-3 species of animals. Examination of pathological and biochemical parameters, including tissue enzyme activities and electron microscopic observations, should be made in order to better understand the potential risk of ingestion of toxaphene. Additional data on the comparative metabolism of toxaphene in various animal species and in man should be gathered so that appropriate animal species can be used in the toxicological evaluations.

The mutagenic potential of toxaphene also requires investigation.

BIBLIOGRAPHY

1. Gosselin, R.E., Hodge, H.C., Smith, R.P. and Gleason, M.N. Clinical Toxicology of Commercial Products. Williams and Wilkins, 4th Ed. 1976.
2. Brooks, G.T. Chlorinated Insecticides, I: Technology and Applications. CRC Press "Uniscience" Series 1: 205, 1974.
3. The Merck Index. Ninth Edition. Merck & Co., Rahway, N.J. : 1821, 1976.
4. Buntin, G.A. U.S. Patent #2565471 Official Gazette :988, Aug. 28, 1951.
5. Casida, J.E., Holmstead, R.L., Khalifa, S., Knox, J.R. and Ohsawa, T. Toxaphene Composition and Metabolism in Rats. Environ. Qual. Saf. 3: 364, 1975.
6. Casida, J.E., Holmstead, R.L., Khalifa, S., Knox, J.R. and Ohsawa, T. Toxaphene Insecticide: A Complex Biodegradable Mixture. Science 183: 520, 1974.
7. Holmstead, R.L., Khalifa, S. and Casida, J.E. Toxaphene Composition Analyzed by Combined Gas Chromatography-Chemical Ionization Mass Spectrometry. J. Agr. Food Chem. 22: 939, 1974.
8. Seiber, J.N., Landrum, P.F., Madden, S.C., Nugent, K.D. and Winterlin, W.L. Isolation and Gas Chromatographic Characterization of Some Toxaphene Components. J. Chromatog. 114: 361, 1975.
9. Landrum, P.F., Pollock, G.A., Seiber, J.N., Hope, H. and Swanson, K.L. Toxaphene Insecticide: Identification and Toxicity of a Dihydrocamphene Component. Chemosphere 5: 63, 1976.
10. Benson, W.R. The Chemistry of Pesticides. Ann. N.Y. Acad. Sci. 160: 7, 1969.
11. Data Sheet on Pesticides. No. 20, Camphechlor. WHO/FAO Publication. VBC/DS/75. 20, 1975.
12. Paris, D.F. and Lewis, D.L. Chemical and Microbioal Degradation of Ten Selected Pesticides in Aquatic Systems. Residue Rev. 45: 95, 1973.
13. Khalifa, S., Holmstead, R.L. and Casida, J.E. Toxaphene Degradation By Iron (II) Protoporphyrin Systems. J. Agr. Food Chem. 24: 277, 1976.

14. Anagnostopoulos, M.L., Parlar, H. and Korte, F. Ecological Chemistry LXXI. Isolation, Identification, and Toxicology of Some Toxaphene Components. Chemosphere 3:65, 1974.

15. Khalifa, S., Mon, T.R., Enge., J.L. and Casida, J.E. Isolation of 2,2,5-endo,6-exo,8,9,10-heptachlorobornane and Octachloro Toxicant From Technical Toxaphene. J. Agr. Food Chem. 22: 653, 1974.

16. Turner, W.V., Khalifa, S. and Casida, J.E. Toxaphene Toxicant A. Mixture of 2,25-endo,6-exo,8,8,9,10-octachlorobornane and 2,2,5-endo,6-exo,8,9,9,10-octachlorobornane. J. Agr. Food Chem. 23: 991, 1975.

17. Howard, R.W., Matsumura, F. and Nelson, J.O. Structure of The Toxic Fraction A of Toxaphene. Chemosphere 4: 271, 1975.

18. Nelson, J.O. and Matsumara, F. Separation and Comparative Toxicity of Toxaphene Components. J. Agr. Food Chem. 23: 984, 1975.

19. Ohsawa, T., Knox, J.R., Khalifa, S. and Casida, J.E. Metabolic Dechlorination of Toxaphene in Rats. J. Agric. Food Chem. 23: 98, 1975.

20. Palmer, K.J., Wong, R.Y., Lundin, R.E., Khalifa, S. and Casida, J.E. Crystal and Molecular Structure of 2,2,5-endo 6-exo,8,9.10-heptachlorobornane, $C_{10}H_{11}Cl_7$, A Toxic Component of Toxaphene Insecticide. J. Am. Chem. Soc. 97:408, 1975.

21. Beckman, H.F., Ibert, E.R., Adams, B.B. and Skoolin, D.O. Determination of Total Chlorine In Pesticides By Reduction With Liquid Anhydrous Ammonia-Sodium Mixture. J. Agr. Food Chem. 6: 104, 1958.

22. Physical and Chemical Properties of Hercules Toxaphene. Tech. Bull. AP-103A, Hercules Corp. (no year available).

23. Graupner, A.J. and Dunn, C.L. Determination of Toxaphene By A Spectraphotometric Diphenylamine Procedure. J. Agr. Food Chem. 8: 286, 1960.

24. Klein, A.K. and Link, J.D. Elimination of Interferences In The Determination of Toxaphene Residues. J.A.O.A.C. 53: 524, 1970.

25. Johnson, D.P. Rapid Test For the Detection of Toxaphene In Agriculture Formulations. J.A.O.A.C. 38: 153, 1955.

26. Hughes, R.A., Veith, G.D. and Lee, G.F. Gas Chromatographic Analysis of Toxaphene In Natural Waters, Fish and Lake Sediments. Water Research, N.Y. 4: 547, 1970.

27. Faucheux, L.J. Jr. Diphenylamine-Zinc Chloride As A Chromogenic Agent For The Detection of A Mixture of DDT, Chlordane, and Toxaphene On Thin Layer Chromatograms. J.A.O.A.C. 48: 955, 1965.

28. Ismail, R.J. and Bonner, F.L. New, Improved Thin Layer Chromatography For Polychlorinated Biphenyls, Toxaphene, and Chlordane Components. J.A.O.A.C. 57: 1026, 1974.

29. Schafer, M.L., Peeler, J.T., Gardner, W.S. and Campbell, J.E. Pesticides in Drinking Water: Water From the Mississippi and Missouri Rivers. Envir. Sci. Tech. 3: 1261, 1969.

30. Terriere, L.C., Kiigemagi, U., Gerlach, A.R. and Borovicka, R.L. The Persistence of Toxaphene In Lake Water and Its Up take By Aquatic Plants and Animals. J. Agr. Food Chem. 14: 66, 1966.

31. Nicholson, H.P., Grzenda, A.R., Lauer, G.J., Cox, W.S. and Teasley, J.L. Water Pollution By Insecticides In an Agricultural River Basin. 1.Occurrence of Insecticides In River and Treated Municipal Water. Limnol. Oceanog. 9: 310, 1964.

32. LaFleur, K.S., Wojeck, G.A. and McCaskill, W.R. Movement of Toxaphene & Fluometuron Through Dunbar Soil To Underlying Ground Water. J. Env. Qual. 2: 515, 1973.

33. Bradley, J.R., Sheets, T.J. and Jackson, M.D. DDT and Toxaphene Movement In Surface Water From Cotton Plots. Environ. Qual. 1: 102, 1972.

34. Ginn, T.M. and Fisher, F.M. Jr. Studies On the Distribution and Flux of Pesticides in Waterways Associated With A Ricefield-Marshland Ecosystem. Pestic. Monit. J. 8: 23, 1974.

35. Zweig, G. and Sherma, J. Toxaphene Analytical Methods For Pesticides and Plant Growth Regulators. G. Zweig and J. Sherma Editors, Academic Press, N.Y. VI: 514, 1972.

36. Terriere, L.C. and Ingalsbe, D.W. Translocation and Residual Action of Soil Insecticides. J. Econ. Entomol. 46: 751, 1953.

37. Hawthorne, J.C., Ford, J.H. and Markin, G.P. Residues of Mirex and Other Chlorinated Pesticides in Commercially Raised Catfish. Bull. Environ. Contam. Toxicol. 11: 258, 1974.

38. Stevens, A.A. and Symons, J.M. Measurement of Organics In Drinking Water. Proceedings AWWA Water Quality Technology Conference, Denver, Colo. XXIII-I-XXIII-25, 1974.

39. Stanley, C.W., Barney, J.E., Helton, M.R. and Yobs, A.R. Measurement of Atmospheric Levels of Pesticides. Environ. Sci. Tech. 5: 430, 1971.

40. Bidleman, T.F. and Olney, C.E. Long Range Transport of Toxaphene Insecticide In The Atmosphere of The Western North Atlantic. Nature 257: 475, 1975.

41. Duke, T.W. Jr. and Wilson, A.J. Jr. Chlorinated Hydrocarbons In Livers of Fishes From The Northeastern Pacific Ocean. Pestic. Monit. J. 5: 228, 1971.

42. Butler, P.A. Organochlorine Residues In Estuarine Mollusks, 1965-72, National Pesticide Monitoring Program. Pestic. Monit. J. 6: 238, 1973.

43. Hughes, R.A. and Lee, G.F. Toxaphene Accumulation In Fish In Treated Lakes For Rough Fish Control. Env. Sci. & Technol. 7: 934, 1973.

44. Hannon, M.R., Greichus, Y.A., Applegate, R.L. and Fox, A.C. Ecological Distribution of Pesticides In Lake Poinsett, South Dakota. Trans. Am. Fish Soc. 99: 496, 1970.

45. Statement of Basis and Purpose For The National Interim Primary Drinking Water Regulations. U.S. Environmental Protection Agency (no date available).

46. Parr, J.F. and Smith, S. Degradation of Toxaphene In Selected Anaerobic Soil Environments. Soil Sci. 121: 52, 1976.

47. Carey, A.E., Wiersma, G.B. and Tai, H. Pesticide Residues In Urban Soils From 14 United States Cities, 1970. Pestic. Monit. J. 10: 54, 1976.

48. Swoboda, A.R., Thomas, G.W., Cady, F.B., Baird, R.W. and Knisel, W.G. Distribution of DDT and Toxaphene In Houston Black Clay On Three Watersheds. Environ. Sci. Technol. 5: 141, 1971.

49. Pharmacologic Properties of Toxaphene, A Chlorinated Hydrocarbon, Insecticide. Med. Arts. & Sci. 7: 4, 1953.

50. Pharmacological Properties of Toxaphene, A Chlorinated Hydrocarbon Insecticide. J.A.M.A. 149: 1135, 1952.

51. Deleted during final draft of report.

52. Nicholson, H.P. Occurence and Significance of Pesticide Residues In Water. J. Wash. Acad. Sci. 59: 77, 1969.

53. Toxaphene. IARC Monograph. WP/77 No. 3, :2601, 1977.

54. Von Rumker, R., Lawless, E.W. and Meiners, A.F. Production, Distribution, Use and Environmental Impact Potential of Selected Pesticides. U.S. Environmental Protection Agency, Washington, D.C. : 196, 1974.

55. Lawrence, J.M. Toxicity of Some New Insecticides To Several Species of Pondfish. Progr. Fish Cult. 12: 141, 1950.

56. Rensberger, B. Pesticide Linked To Defects In Fish. New York Times, April 10, : 8, 1975.

57. LaFleur, K.S. Toxaphene-Soil-Solvent Interactions. Soil Science 117: 205, 1974.

58. Durant, C.J. and Reimold, R.J. Effects of Estuarine Dredging of Toxaphene Contaminated Sediments In Terry Creek, Brunswick, Ga.-1971. Pestic. Monit. J. 6: 94, 1972.

59. Reimold, R.J. and Durant, C.J. Toxaphene Content of Estuarine Fauna and Flora Before, During and After Dredging Toxaphene-Contaminated Sediments. Pestic. Monit. J. 8: 44, 1974.

60. Reimold, R.J. amd Shealy, M.H. Jr. Residues In Fish Wildlife, and Estuaries: Chlorinated Hydrocarbon Pesticides and Mercury In Coastal Young-Of-The-Year Finfish, South Carolina and Georgia 1972-74. Pestic. Monit. J. 9: 170, 1976.

61. Cohen, J.M., Rourke, G.A. and Woodward, R.L. Effects of Fish Poisons On Water Supplies. J. Am. Water Works Assoc. 53: 49, 1961.

62. Young, L.A. and Nicholson, H.P. Stream Pollution Resulting From The Use of Organic Insecticides. Progr. Fish Cult. 13: 193, 1951.

63. Hooper, F.F. and Grzenda, A.R. The Use of Toxaphene As A Fish Poison. Trans. Am. Fish Soc. 85: 180, 1957.

64. Hamphill, J.E. Toxaphene As A Fish Toxin. Progr. Fish Cult. 16: 41, 1954.

65. Yapp, H.H., Desaiah, D., Cutkomp, L.K. and Koch, R.B. In Vitro Inibition of Fish Brain ATPase Activity By Cyclodiene Insecticides and Related Compounds. Bull. Environ. Contam. Toxicol. 14. 163, 1975.

66. Barthel, W.F., Parsons, D.A., McDowell, L.L. and Grissinger, E.H. Surface Hydrology and Pesticides. A Preliminary Report on the Analysis of Sediments of the Lower Mississippi River. Symposium on Pesticides and Their Effect on Soil and Water: 128, 1965.

67. Kallman, B.J., Cope, O.B. and Navarre, R.J. Distribution and Detoxication of Toxaphene in Clayton Lake, New Mexico. Trans. Am. Fish Soc. 91: 14, 1962.

68. Archer, T.E. Toxaphene Residues On Alfalfa Hay Exposed To Drying By Sunlight, Ultraviolet Light, and Air. J. Dairy Sci. 54: 1180, 1971.

69. 1968 Evaluations of Some Pesticide Residues in Food. FAO/PL: 1968/M/9/1, WHO Food Add. /69.35.

70. Menzie, C.M. Fate of Pesticides in the Environment. Ann. Rev. Entomol. 17: 199, 1972.

71. Alexander, M. Persistence and Biological Reactions of Pesticides in Soils. Soil Sci. Soc. Am. Proc. 29: 1, 1965.

72. Nash, R.G. and Woolson, E.A. Persistence of Chlorinated Hydrocarbon Insecticides in Soils. Science 157: 924, 1967.

73. Veith, G.D. and Lee, G.F. Water Chemistry of Toxaphene - Role of Lake Sediments. Environ. Sci. Technol. 5: 230, 1971.

74. Durant, C.J. and Reimold, R.J. Effects of Estuarine Dredging of Toxaphene-Contaminated Sediments In Terry Creek, Brunswick, Ga., 1971. Pestic. Monit. J. 6: 94, 1972.

75. Newsom, L.D. Consequences of Insecticide Use on Non-target Organisms. Ann. Rev. Entomol. 12: 257, 1967.

76. Quality Criteria For Water - Toxaphene. Environmental Protection Agency, 1976.

77. Sanborn, J.R., Metcalf, R.L., Bruce, W.N. and Lu, P. The Fate of Chlordane and Toxaphene In a Terrestrial-Aquatic Model Ecosystem. Environ. Entomol. 5: 533, 1976.

78. Barlow, F. and Hadaway, A.B. Studies on Aqueous Suspensions of Insecticides. V. The Sorption of Insecticides By Soils. Bull. Entomol. Res. 46: 547, 1956.

79. Fowkes, F.M., Benesi, H.A., Ryland, L.B., Sawyer, W.M. and Detling, K.D. Clay-Catalyzed Decomposition of Insecticides. J. Agr. Food Chem. 8: 203, 1960.

80. Muirhead-Thomson, R.C. Toxaphene: The Elimination of Entire Fish Populations. "Pesticides and Freshwater Fauna" Academic Press, N.Y., N.Y. : 72, 1971.

81. Preliminary Assessment of Suspected Carcinogens in Drinking Water. Report to Congress. U.S. Environmental Protection Agency, Dec. 1975.

82. Smillie, R.D., Nicholson, A.A., Meresz, O., Duholke, W.K. and Rees, G.A.V. Organics In Ontario Drinking Waters Part II - A Survey of Selected Water Treatment Plants. Ontario Ministry of The Environment, OTC 7703, April, 1977.

83. Coffin, D.E. and McLeod, H.A. Current Pesticide Residue Status of Canadian Diets and Adipose Tissues. 7th Eastern Canadian Symposium on Pesticide Residue Analysis, Toronto, May 20, 1975.

84. Smith, D.C. Pesticide Residues In The Total Diet In Canada. Pestic. Sci. 2: 92, 1971.

85. Smith, D.C., Sandi, E. and Leduc, R. Pesticide Residues In The Total Diet In Canada. II-1970. Pestic. Sci. 3: 207, 1972.

86. Smith, D.C., Leduc, R. and Charbonneau, C. Pesticide Residues In The Total Diet In Canada. III-1971. Pestic. Sci. 4: 211, 1973.

87. Claborn, H.V., Mann, H.D., Ivey, M.C., Radeleff, R.D. and Woodard, G.T. Excretion of Toxaphene and Strobene In The Milk of Dairy Cows. J. Agr. Food Chem. II: 286, 1963.

88. Arthur, R.D., Cain, J.D. and Barrentine, B.F. Atmospheric Levels of Pesticides In The Mississippi Delta. Bull. Environ. Contam. Toxicol. 15: 129, 1976.

89. Mehrle, P.M., Mayer, F.L. and Foster, L. Toxaphene Effects On Growth and Bone Composition of Fathead Minnows Pimephales Promelas. J. Fish Res. Bd. Canada J. 32: 593, 1975.

90. Crowder, L.A. and Dindal, E.F. Fate of ^{36}Cl-Toxaphene In Rats. Bull. Environ. Contam. Toxicol. 12:320, 1974.

91. Schaper, R.A. and Crowder, L.A. Uptake of ^{36}Cl-Toxaphene In Mosquito Fish, Gambusia Affinis. Bull. Environ. Contam. Toxicol. 15: 581, 1976.

92. Lackey, R.W. Observations On The Acute and Chronic Toxicity of Toxaphene In The Dog. Ind. Hyg. & Tox. J. 31: 117, 1949.

93. Henderson, C., Pickering, Q.H. and Tarzwell, C.M. Relative Toxicity of Ten Chlorinated Hydrocarbon Insecticides To Four Species of Fish. Trans. Am. Fish Soc. 88: 23, 1959.

94. Gaines, T.B. Acute Toxicity of Pesticides. Toxicol. Appl. Pharmacol. 14: 515, 1969.

95. McGee, L.C., Reed, H.L. and Fleming, J.P. Accidental Poisoning By Toxaphene. Review of Toxicology and Case Reports. J.A.M.A. 149: 1124, 1952.

96. Boyd, E.M. and Taylor, F.I. Toxaphene Toxicity In Protein-Deficient Rats. Toxicol. Appl. Pharmacol. 18: 158, 1971.

97. Luckens, M.M. Comparative Toxicologic Studies of Selected Organochlorine Pesticides In Native and Laboratory Mice. Toxicol. Appl. Pharmacol. 17: 277, 1970.

98. O'Brien, R.D. Cyclodienes. Insecticides: Action and Metabolism, Academic Press, N.Y :136, 1967.

99. Schwartz, C.C., Nagy, J.G. and Streeter, C.L. Pesticide Effect On Rumen Microbial Function. J. Animal Sci. 37: 821, 1973.

100. Schwartz, C.C. and Nagy, J.G. Pesticide Effects On *In Vitro* Dry Matter Digestion In Deer. J. Wildl. Manage. 38: 531,1975.

101. Radeleff, R.O. Insecticides, Acaricides & Antihelmintics. Veterinary Toxicology. 2nd Ed. Lea & Febiger, Philadelphia, Pa. 243: 238, 1970.

102. Criteria Document For Toxaphene. Environmental Protection Agency Publication. PB 253: 677, 1976.

103. Atallah, Y.H. and Newsom, L.D. Ecological and Nutritional Studies On Coleomegilla Maculata. III. The Effect of DDT, Toxaphene and Endrin On The Reproductive and Survival Potentials. J. Econ. Entomol. 59: 1181, 1966.

104. Wolfenbarger, D.A. Synergism of Toxaphene-DDT Mixtures Applied Topically To The Bollworm and The Tobacco Budworm. J. Econ. Entomol. 66: 523, 1973.

105. Adkisson, P.L. Development of Resistance By The Tobacco Budworm To Mixture of Toxaphene or Strobane Plus DDT. J. Econ. Entomol. 60: 788, 1967.

106. McGarr, R.L. and Wolfenbarger, D.A. Methyl Parathion, Toxaphene, and DDT used Alone and In Combination For Control of Several Cotton-Insects. J. Econ. Entomol. 62: 1249, 1969.

107. Sanders, H.O. and Cope, O.B. The Relative Toxicities of Several Pesticides To Naiads of Three Species of Stoneflies. Limnol. Oceanog. 13: 112, 1968.

108. Koeppe, R. The Toxicology and Toxicity of Toxaphene With Respect To Fish and Aquatic Food Animals. Z. Fisch. 9: 771, 1962.

109. Macek, K.J. and McAllister, W.A. Insecticide Susceptibility of Some Common Fish Family Representatives. Trans. Amer. Fish Soc. 1: 20, 1970.

110. Chaiyarach, S., Ratananun, V. and Harrel, R.C. Acute Toxicity of The Insecticides Toxaphene and Carbaryl and The Herbicides Propanil and Molinate To Four Species of Aquatic Organisms. Bull. Environ. Contam. Toxicol. 14: 281, 1975.

111. Duodoroff, P., Katz, M. and Tarzwell, C.M. Toxicity of Some Organic Insecticides To Fish. Sewage and Ind. Wastes 25: 840, 1953.

112. Stadnyk, L., Campbell, R.S. and Johnson, B.T. Pesticide Effect On Growth and ^{14}C Assimilation In A Freshwater Algae. Bull. Environ. Contam. Toxicol. 6: 1, 1971.

113. Ukeles, R. Growth of Pure Cultures of Marine Phytoplankton In The Presence of Toxicants. Appl. Microbiol. 10: 532, 1962.

114. Hoffman, D.A. and Olive, J.R. The Effects of Rotenone and Toxaphene Upon Plankton of Two Colorado Reservoirs. Limnol. Oceanogr. 6: 219, 1961.

115. Cullinan, F.P. Some New Insecticides - Their Effects On Plants and Soil. J. Econ. Entomol. 42: 287, 1949.

116. Kralovic, J. Effect of Some Insecticides On The Photosynthesis of Alfalfa. Environ. Qual. Saf. 3: 554, 1975.

117. Johnston, B.L. and Eden, W.G. The Toxicity, of Aldrin, Dieldrin, and Toxaphene To Rabbits By Skin Absorption. J. Economic Entomol. 46: 702, 1953.

118. Lackey, R.W. Observations On The Percutaneous Absorption of Toxaphene In The Rabbit and Dog. II J. Indus. Hyg. Toxicol. 31: 155, 1949.

119. Welch, R.M., Levin, W., Kuntzman, R., Jacobson, M. and Conney, A.H. Effect of Halogenated Hydrocarbon Insecticides On The Metabolism and Uterotropic Action of Estrogens in Rats and Mice. Toxicol. Appl. Pharmacol. 19: 234, 1971.

120. Genelly, R.E. and Rudd, R.L. Chronic Toxicity of DDT, Toxaphene and Dieldrin to Ring-Necked Pheasants. Calif. Fish Game, 42: 5, 1956.

121. Arscott, G.H., Michener, S. and Bills, D.D. Effect of Toxaphene On The Performance of White Leghorn Layers and Their Progeny. Poult. Sci. 55: 1130, 1976.

122. Genelly, R.E. and Rudd, R.L. Effects of DDT, Toxaphene and Dieldrin On Pheasant Reproduction. Auk. 73: 529, 1956.

123. Ortega, P., Hayes, W.J. and Durham, W.F. Pathologic Changes In The Liver of Rats After Feeding Low Levels of Various Insecticides. A.M.A. Arch. Pathol. 64: 614, 1951.

124. Kennedy, G.L., Frawley, J.P. and Calandra, J.C. Multigeneration Reproductive Effects On Three Pesticides In Rats. Toxicol. Appl. Pharmacol. 25: 589, 1973.

125. Keplinger, M.L., Diechmann, W.B. and Sala, F. Effects of Combinations of Pesticides on Reproduction in Mice. Ind. Med. Surg. 37: 525, 1968.

126. Lowe, J.I. Chronic Exposure of Spot, Leiostomus Xanthurus, To Sublethal Concentrations of Toxaphene in Seawater. Am. Fish Soc. Trans. 93: 396, 1964.

127. Lackey, R.W. and Weed, O. Treatment of Acute Toxaphene Poisoning In The Dog. Southern Med. J. 44: 165, 1951.

128. McWhorter, G.M., Berry, E.C. and Robinson, J.F. Field Persistence of Six Insecticides For European Corn Borer Control. J. Econ. Entomol. 69: 419, 1976.

129. Surber, E.W. Chemical Control Agents and Their Effects On Fish. Prog. Fish Cult. 10: 125, 1948.

130. Korn, S. and Earnest, R. Acute Toxicity of Twenty Insecticides To Striped Bass, Morone Saxatilis. Calif. Fish and Game 60: 128, 1974.

131. Lehman, A.J. Chemicals In Food. A Report To The Association of Food & Drug Officials. Assoc. of Food & Drug Officials, 16: 47, 1952.

132. Fitzhugh, O.G. and Nelson, A.A. Comparison of Chronic Effects Produced In Rats By Several Chlorinated Hydrocarbon Insecticides. Fed. Proc. 10: 295, 1951.

133. Fouts, J.R. and Hart, L.G. Hepatic Drug Metabolism During The Perinatal Period. N.Y. Acad. Sci. Ann. 123: 245, 1965.

134. Kinoshita, F.K., Frawley, J.P. and DuBois, K.P. Quantitative Measurement of Induction of Hepatic Microsomal Enzymes By Various Dietary Levels of DDT and Toxaphene In Rats. Toxic. Appl. Pharmacol. 9: 505, 1966.

135. Peakall, D.B. Effects of Toxaphene On Hepatic Enzyme Induction and Circulating Steroid Levels In The Rat. Environ. Health Perspect. 13: 117, 1976.

136. Kuzminskaya, U.A. and Alekhina, S.M. Effect of Chlorocamphene On The Isoenzyme Spectrum of Lactate Dehydrogenase In Rat Serum and Liver. Environ. Health Perspect. 13: 127, 1976.

137. Pardini, R.S., Heidker, J.C. and Payne, B. The Effect Of Some Cyclodiene Pesticides, Benzenehexachloride and Toxaphene on Mitochondrial Electron Transport. Bull. Environ. Contam. Toxicol. 6: 436, 1971.

138. Bengtsson, B.E. Vertebral Damage In Fish Induced By Pollutants. Sublethal Effects of Toxic Chemicals On Aquatic Animals. Koeman, J.H. and Strik, J.J., Editors. Amsterdam, Elsevier Scientific, WA 689 S941 :23, 1975.

139. Desaiah, D and Koch, R.B. Toxaphene Inhibition of ATPase Activity In Catfish, Ictalurus, Punctatus, Tissues. Bull. Environ. Contam. Toxicol. 13: 238, 1975.

140. Rutschke, E. and Brozio, F. The Influence of Toxaphene On Hepatic Cells Of The Carp (Cyprinus Carpio L.) Biol. Zentralbl. 94: 441, 1975.

141. Pesticide Residues In Food. Technical Report Series 545 WHO, 1974.

142. Chernoff, N. and Carver, B.D. Fetal Toxicity of Toxaphene In Rats and Mice. Bull. Environ. Contam. Toxicol. 15: 660, 1976.

143. Keplinger, M.L. and Diechmann, W.B. Acute Toxicity of Combinations of Pesticides. Toxicol. Appl. Pharmacol. 10: 586, 1967.

144. Macek, J. Acute Toxicity of Pesticide Mixtures To Bluegills. Bull. Environ. Contam. Toxicol. 14: 648, 1975.

145. Haun, E.C. and Cueto, C. Jr. Fatal Toxaphene Poisoning In A Nine Month Old Infant. Am. J. Dis. Child. 113: 616, 1967.

146. Pollock, R.W. Toxaphene Poisoning - Report of a Fatal Case. Northwest Med. 52: 293, 1953.

147. Hartfield, I. Fly Control Chemicals & Toxicity. Pests. 17: 9, 1949.

148. Conley, B.E. Pharmacologic Properties of Toxaphene, A Chlorinated Hydrocarbon Insecticide. J.A.M.A. 149: 1135, 1952.

149. Morgan, D.P. Recognition and Management of Pesticide Poisonings. U.S. Environmental Protection Agency. Office of Pesticide Programs. August, 1976.

150. Wauraki, S. Respiratory Hazards of Chlorinated Camphene. Arch. Environ. Health 7: 253, 1963.

151. Yoder, J., Watson, M. and Benson, W.W. Lymphocyte Chromosone Analysis of Agricultural Workers During Extensive Occupational Exposure to Pesticides. Mutation Res. 21: 335, 1973.

152. Parker, W.L. and Beacher, J.H. Toxaphene, A Chlorinated Hydrocarbon With Insecticidal Properties. Dela. Agr. Enpt. Sta. Bull. 264: 1947.

153. Kadis, V.W., Breitkreitz, W.E. and Jonasson, O.J. Insecticide Levels In Human Tissues Of Alberta Residents. Can. J. Public Health 61: 413, 1970.

154. Weil, C.S. Statistics vs. Safety Factors and Scientific Judgement In The Evaluation of Safety For Man. Toxicol. Appl. Pharmacol. 21: 454, 1972.

155. Mantel, N. and Bryan, W.R. "Safety" Testing of Carcinogenic Agents. J. Nat. Cancer Inst. 27: 455, 1961.